主論文1.

The Breeding Structure of the Three-spined Stickleback, *Gasterosteus aculeatus* (forma *leiura*) with reference to Spatial and Temporal Patterns of Nesting Activity

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1. Introduction

One of the most important knowledges to understand the ecology of animals is how individual allocate time and space among reproductive activities in the face of environmental variablity. Individual should have a pattern of allcation which maximizes the number of offspring produced in the lifetime. The pattern of allocation of time and space will be strongly related to the breeding system. So far, the breeding system, which is closely connected with reproductive success, has been intensively described and studied in various animals. However, precise studies of the ecological significance of the combined use of time and space related to animal reproduction are in their infancy situation.

Recently, variability in a specific behaviour has been examined with particular reference to mating strategies or breeding systems (see Krebs and Davies, 1978; Clutton-Brock, 1988). This variablity must bring about individual differences in reproductive success. Heretofore, it has been frequently reported that in natural population of various animals there was a great deal of variations in individual reproductive traits (e. g., timing of breeding initiation, quality of occupied breeding sites, breeding or mating behaviour and mating frequency), in breeding situations in which individuals were (density, distribution, abundance of breeding individuals, sex ratio, predation pressure and food resource) and in morphological and physiological traits (body shape, body size, nuptial colour, sexual characteristics, age, sex and growth). Furthermore it is also important to notice that the reproductive variations which fluctuate with time progressing may

be risen as a consequence of environmental changes (see Barnard, 1983; Bateson, 1983; Alcock, 1984; Trivers, 1985).

However, most of those studies have largely treated samples of data collected on the basis of activities of different individuals at particular areas in a given short time. Very few studies deliberately followed definite individuals at a given observation time per day. The selection and adaptation argued in those most studies were commonly estimated by estimates of fitness measured over a small fraction of an individual's life span. Such estimates of fitness founded on short-term data without identifying an individual can easily be misread for example by confined results of single or unusual episodes of selection (i. e., ecological noise). Therefore, reproductive success of individual animals must be tracked through most or all of their natural life span, else over the whole breeding season. And then, considering the above methodological problem, we need to make improvements in the study of the following views.

Reproductive success intensely depends on when and where individual reproduces and on the resources it allocates to reproduction. In one word, a study of the reproductive ecology is mostly related to when and where breeding activities take place and what resources are allocated to reproduction. This problem raises that how much of the variance in reproductive success is contributed by the different components of breeding timing and spacing of individual. The extent and causes of individual differences in reproductive success have been still rudimentary states in studies of natural populations (e. g., Clutton-Brock, 1988).

In studies of fish comparing with those of other animals, individual differences in reproductive success have been probably hardly

researched through the whole breeding season or lifetime. Kodoric-Brown (1977) and Katano (1990) noticed that there was an individual variation in reproductive success in freshwater fish. However, little is known of the relationship between the variation and the temporal and spatial aspects of breeding system under the prevailing environmental conditions. Furthermore, there are few quantitative studies with respect to the effect of social interaction on individual reproductive success.

The three-spined stickleback, *Gasterosteus aculeatus*, is one of the most popular experimental fish and has been studied particularly its ethology, physiology and morphology for many years, (see Wootton, 1976, 1984). These studies have provided considerable information on the breeding biology of the fish in relation to aggressive, courtship and parental behaviours, and formed the basis for the present study. It has been traditionally regarded that the three-spined stickleback is a small fish that is generally capable of reproducing at a relatively early age and has a short life-span. The fish commonly breeds only late in life spend most of the time on the breeding ground during the breeding season and most fish die either while breeding or soon after.

In this study, I examined systematically in detail the long-term breeding system of the three-spined stickleback which has been under study for several years (Mori, 1985). There are a number of factors which determine and influence reproductive success. Heretofore, it has been stated on the strength of laboratory experiments that reproductive success would be simply related to fish density, territory size and nest location (van den Assem, 1967; Black, 1971; Kynard, 1978; Sargent and Gebler, 1980; FitzGerald and Whoriskey,

1985; Whoriskey and FitzGerald, 1985a). Here, I proposed not only reexamination for each factor as mentioned above and much more additional factors but also synthetical evaluation for them. Mainly, the present study would investigate (a) the effect of environmental variablity on reproductive success, (b) the various factors of temporal and spatial nest distribution in relation to mating opportunities, aggression against intruders and predation and (c) the advantageous balance of associative nesting pattern which was adopted as the neighbouring synchronous nesting linkage.

2. Study area and methods

2-1. Study area

This study was performed in one of small inlet stream in the Tsuya River (about 16 km long, 35° 09' N, 136° 34' E, 5 m above sea level), flowing lastly into Pacific Ocean, in Central Japan (Fig. 1). The current of the river is very slow and belongs to entirely Bc type according to the claasification of streams proposed by Kani (1944; also see Mizuno and Kawanabe, 1981). The river has abundant inlets from springs along the right shoreline (Mori, 1985, 1987b, c).

Main study area was in a part of one of the inlets (63 m in length, 2.7 m in width and 35 cm - 84 cm in depth) with a horizontal terrace (110 cm in width) (Fig. 2). Both shores had been protected by concrete plates in 1981 (Plate I), and its substrate was uniformly consisted of mud. Fourty eight piles made of concrete are set equally distant (140 cm) along the bank of the entire inlet. Pile number 0 was located at the upper end of the inlet.

Water temperatures in the main stream and in the inlet stream during the study period are given in Figure 3. The temperature of the main stream varied between 7°C and 25°C during the study period. On the other hand, the inlet had a constant temperature of 15°C all through the year (Mori, 1985). The constant temperature of the inlet resulted from the unexhausted inflow of spring water. As a rule, in April and October the temperature of the main stream was similar (15°C) to that of the inlet.

Daily I measured water depth at a fixed point in the terrace. The water level ranged from 12 cm under the terrace level to 75 cm above during the study period (Fig. 4). The velocity at the study pool was relatively constant and seldom exceeded 15 cm/sec (usually 4-10 cm/sec).

In the inlet, the submerged plants were patchily distributed and consisted of *Elodea, Myriophyllum, Nasturtium and Vallisneria*. In the enclosure pool *Elodea* dominated during the study period (Fig. 5). The bottom area of the enclosure pool which was covered with vegetation increased during the course of the season. In June tripton was considerably abundant in the upper end of the study pool (pile numbers 0-3). In the enclosure pool abundant amounts of food were available, and the availability was relatively constant throughout the year (Mori, in preparation).

There were rarely the fatminnow (*Phoxinus lagowski*, 4-10 cm in body length), young of the rose bitterling (*Rhodeus ocellatus*, 3-5 cm), the floating goby (*Chaenogobius urotaenia*), the tadpoles (*Rana*) and crawfish (*Procambarus*) in the enclosure pool. These animals which coexisted with the three-spined stickleback most likely had no influence on nesting activities of sticklebacks, because their numbers

were low and they did not interact with sticklebacks. Stomach analysis of the floating goby showed, however, that they sometimes fed on stickleback fry. After late May larger numbers of tadpole larvae appeared in the inlet including the enclosure pool. Although the tadpoles were large (5-10 cm) and fast swimming, they rarely caused destruction of nests. As they were herbivorous, they formed no danger to eat either stickleback's own eggs or fry.

2-2. Methods

The main study was made almost daily from March 7th to July 14th 1988. At night of 5th March, I enclosed the upper part of the stream, including 1 piles, with a 150 cm high net fence (about 2 mm in mesh size, Fig. 2). The topography of the enclosure pool was mapped in detail. At the enclosing time there were 5 nests which I collected and were excluded from the data.

All the males which nested and most females were individually tagged with coloured tube slipped over the 1st and/or 2nd dorsal spines. The tags had no influence on nesting success (= hatching), dates of first nest-building initiation (Appendix I) and nest-site types (ANOVA, $p>0.3$). Marking was mainly performed at night between 19:00 and 4:00. Tags were replaced every 2-6 weeks, but I stopped the marking for male in the last 3 weeks, because the fish could be identified by individual differences in colour pattern of the dorsal and lateral sides of the body, body length as measured by eye as well as body shape. I sketched the pattern of the black pigmented areas on the left side of each of the fish (Appendix II) and the pattern remained unchanged during the season, though the depth of black changed.

Each nest was also indentified by bamboo sticks (about 20 cm long, 2

mm in diameter) planted in the substratum next to the nest (Plate II). Of all fish the body length was measured at the date of first capture (nearest 1 mm). During the whole season dead fish (16 males and 8 females) were collected and examined on the cause of death.

Fish were usually observed from 1.5 - 2 m above the surface and sometimes under water with the aid of a face plate and snorkel. The following 8 information was collected from each nest, male and female.

a) Nest location.

Water depth, distance from the shore, distance to the nearest nest, nest density within a radius of 50 cm and 100 cm of the nest, the degree of cover, and proportion of algal cover within a radius of 50 cm were observed (Fig. 6). The water depth at each nest was measured during the nest stages II to IV. Cover by submerged water vegetations was classified as 0 %: cover absent; 25 %: nest covered on one side; 50 %: nest covered on two sides; 75 %: nest covered on three sides; and 100 %: nest entirely in cover but visible to an overhead observer (see FitzGerald, 1983). For convenience, nest density was also calculated in each section between numbered piles.

b) Male nesting activity.

Date of nest-building initiation (DNI), estimated date of laying first eggs, hatching date, fledging date (when fry dispersed around the nest while the male no longer showed parental care, and only brief nest directed behaviour), date of nesting end, and interval days till the next nesting were checked (Fig. 7), and first egg-laying date was estimated from hatching date. Actual nest days (AND) was defined as the number of days in which the nest contained eggs (incubation

period, ID) plus the number of days in which the male cared for the fry (fry caring period, CD), called parental phase in the previous literature. Distance (cm) and direction between successive nests were also recorded.

Besides, I recorded once a week the number of nests and hatching dates in the whole inlet to determine the breeding peak in an unenclosed natural situation. This recording was made to examine whether the breeding conditions in the enclosure pool deviated from the natural situation. I confirmed with recordings once a week the fluctuations of nest positions and nest numbers until December 1988.

The chronology of nests in the 1986 and 1987 data will be additionally provided for comparison with this study. I have visited this study area at least twice a month since February 1986.

Because of the constant temperature in the enclosure pool, temperature effects on nesting activity and breeding success are ruled out.

c) Nest stages.

Description of the nesting process were followed by Wunder (1930) and van Iersel (1953), as follows. (I) A well-defined nest entrance and a steady roof covered with or without sand is present. The nest does not contain eggs. (II) The male makes some small holes in the roof and around the nest. (III) Large holes are made in the roof and the male predominately fans before the nest entrance for ventilating the eggs. (IV) The nest apart leaving a part of nest material in the nest pit with recently hatched fry which lie in the tangled mass of vegetation, and any fry moving away from the nest is sucked into the male's mouth and spit out into the nest. (V) Large distinct holes

are there over the nest and the fry begin to scatter around the rim of the nest. (VI) The nest gets in considerably coarse condition with scattered fry around the nest, and the nesting male has decreased the amount of fanning. (VII) The fry almost disappears from the nest. The male sometimes maintains nesting only for one to three days, and then abandons the nest.

d) Nuptial coloration.

Variation in the intensity of nuptial coloration was scored with the aid of a 7-point scale as shown in Table 1. The brightest males were given a score of V. Males with lower and higher scores than V had a duller appearance.

e) Male breeding behaviours.

Observations were usually made between 10:00 and 16:00 from a 1.2 - 2.0 m distance from an observer on the terrace of the enclosure pool bank. Each nesting male was one to three times daily the focus fish for 5 minutes. According to Ward and FitzGerald (1987) this observation period was sufficient to quantify the behavioural reperton of the focus fish. The behaviour of some males in three selected areas was recorded for 10 minutes to one hour per day. These areas were three pile sections (0-2, 3-4 and 9-10) which had many nests. To obtain data on the time budgets of nesting males, I observed 99 males which included successful (i. e. males that produced offspring) and unsuccessful individuals. Behaviours were as expressed as duration and/or frequencies. I recorded the following behaviour items, of which a detail description can be found in Wootton (1976):

1) Swimming route: route that a male swims for 5 minutes. It was

drawn on a topographical map which was made in advance on a section paper.

2) Stay at nest: time at which the male stays at or within 10 cm from the nest per 10 minutes.

3) Nest-directed activities: pushing the nest, boring the substrate, glueing nest materials, sucking sand and creeping through the nest.

4) Fanning: entering fresh water into the nest with movements of the pectoral fins.

5) Courtship: a courting male approaches a female with a series of zig-zag movements.

6) Aggression: chases, lunges, bites, threat displays directed toward male and female conspecifics and heterospecifics.

7) Intruder: frequency of territorial intrusions and the routes that conspecific males and females follow which intruded upon the territory. The response of the territory owner to the intruders was recorded.

8) Territory size: territory size was determined by the swimming route and points where aggressive behaviours were shown. The size was calculated from drawing of the swimming route on section paper. One section represented an area of 5 square cr Territory size was averaged over the different phases (sexual and parental phase) because during the parental phase it tends to decrease (Black, 1971; Mori, in preparation).

9) Home range size: this area was determined by the swimming route regardless of aggressive responses. The presence and significance of home range will be described in detail elsewhere.

f) Nest density.

The density and the distributional patterns of nests were analysed by I_δ-method (Morisita, 1959):

$$I_\delta = q \cdot \frac{\sum_{i=1}^{q} X_i(X_i-1)}{N(N-1)} \quad , \quad N = \sum_{i=1}^{q} X_i$$

q = the number of quadrat, Xi = the number of individuals per quadrat. The distributional pattern of nest was analysed by the curve of I - index to quadrat square (Morisita, 1959). This analysis was also made in the whole inlet stream in 1986 and 1987. Besides, I counted the number of nests within a radius of 50 cm and 100 cm from the nest at the nest stage III and V, these being the best representations of a stickleback's choice of a nesting site in relation to its neighbours. Minimum inter-nest distance was considered as the distance to the nearest neighbour.

g) Sex ratio.

The sex ratios were assessed by counting fish collected four times in the enclosure during the study period. I counted the numbers of nesting males, wandering males, swollen females and spawned females. At the start of the preliminary study (February 2, 1988), there were 83 females and about 42 % of them was gravid in the pool. The male-biased sex ratio (nuptial coloured males : gravid female = 2 : 1) occurred at the beginning of the breeding season, but later in the season the reverse happened to be true. The operational sex ratio (nesting male : gravid female) showed great alternations during the study period (see results). Females were assessed their degree of ripeness. Most females were sexually mature adults (mature grades IV-

V; Nikolsky, 1963), with a body size ranging from 46 to 57 mm at the beginning of the season. Some had a mature grade III and were not ready to spawn.

h) Reproductive success.

To determine reproductive success I collected (for all nests and all all nest owners), the following data during the study period: 1) nesting success whether young hatched from the nest; 2) date of hatching; 3) date of first egg laying estimated from the date of hatching; 4) the number of nesting males; 5) unsuccessful and successful nesting times per male; 6) the number of eggs per nest, which were classified by scores I-VI (RS-index). The RS-index was calculated on the basis of three degrees of which the hatched fry distributed in and around the nest on a given date. The three degrees (1, 2, and 3) represented the amount of fry less than 50 at the nest, from 50 to 200 fry and fry more than 200, respectively, on eye measurement. The scores (I-VI) were calculated as the following: $\sum_{n=1}^{5} Xn$, (Xn = the degree at every other day n; n<5, day 1 = first hatching date). These data were used to estimate reproductive success, i. e., the number of an individual's progeny surviving to reproduce in the future generation (Trivers, 1985).

3. Breeding biology

The reproduction of the three-spined stickleback is characterized by male territoriality, nest building, courtship display and parental care by the male (ter Pelkwijk and Tinbergen, 1937; Tinbergen, 1951;

van Iersel, 1953; Wootton, 1976). Males aggressively defend a breeding territory during the breeding season. After establishing the territory and building a nest, they court one or several ripe females, and then provide parental care to their eggs and fry. They have the potential for more than one breeding cycle per breeding season (Wootton, 1976, 1984). On the other hand, females are usually free to forage for food apart from during courtship and spawning. During a single breeding season, they have the potential of producing several clutches, which depends strongly on the food supply (Allen & Wootton, 1982a, b; Wootton, 1974, 1977; Wootton, et al. 1978).

The first two sections of this chapter provide the breeding characteristics of the population in the Tsuya waterbodies on the basis of investigations in the inlet from 1986 to 1988, while the later sections are based on data obtained in 1988.

3-1. Breeding migration into the inlet.

Figure 8 gives the monthly changes in the numbers of adults fish present in different parts of the inlet and in the river. During autumn and winter most sticklebacks inhabited the Tsuya River and then were also present in the vicinity of the mouth of the inlet, though some schools of immature fish (about 200-400 fish, 28-43 mm in BL) were present in the lower part of the inlet.

Before the beginning of February, almost no adult fish were present in the upper part of the inlet (the future enclosure pool). In the middle of February many mature fish began to migrate in to the upper part of the inlet and the numbers increased considerably, followed by a more gradual increase thereafter. On 8th March, I found two nests and many matured fish in the upper part of the inlet stream which I

enclosed afterwards. In April, a relatively small number of fish was caught in the upper part the inlet. This has to be ascribed to migration into the creek area which is located more upstream than the future enclosure pool. Additionally, I have observed such a migration pattern since 1986.

Most females tended to arrive later at the breeding sites than the males. There were few females avaiable at the breeding sites in January and February, though some of them were already ripe. The differed migrating behaviour of males and females had an effect on the operational sex ratio at the breeding grounds (see section 3-7).

3-2. Breeding period

In Central Japan the breeding period of the freshwater three-spined stickleback (forma *leiura*) tends to be prolonged through the whole year with a peak during April and mid May (Mori, 1985, 1986). Figure 9 gives the temporal distribution of nests in the whole inlet from 1986 to 1988 (included the enclosure pool). In all three years, there was a marked peak of nest-building in April, followed by a second small peak in October (Mori, 1985, in preparation). The first deduced from data on body lengths which were collected monthly all through the year (Mori, unpublished data).

In 1988, breeding activities were first seen in early January, and continued to late December (Fig. 9). The number of nests sharply increased in March and declined in June, like the pattern of breeding activity in 1986 and 1987. In order to know whether the pattern in the enclosure pool is natural, I compared it with that in the inlet. It turned out that the pattern of breeding activity in the enclosure pool was almost the same as in the whole inlet.

After the study period the fish in the enclosure pool were caught and I compared the relative gonad and the kidney weight between territorial and non-territorial males. These differences were not statistically in significant (ANOVA, $p>0.1$). The gonad weight of males was about 0.5 % of the total body weight.

3-3. Nest site location in relation to vegetation

The places selected for nesting were strikingly similar with respect to vegetation. There was a strong preference for bare muddy places at the borders of vegetation (Fig. 5). The majority of males (163/189, 86.2 %) nested where vegetation or the shore was located within a 30 cm radius of nest (X2-test, $p<0.001$). Although the chosen area might be partly covered with vegetation, the males clearly avoided nesting in areas where vegetation was too abundant. The late breeders usually started nest-building in a period in which the vegetation was in advanced stage of development.

As the number of available sites strongly decreased during the course of the season (they were either occupied by the early nesting males or overgrown by vegetation), the late breeders were very limited in their choice. Although they also first tried to select the places along the shore or vegetation, they had no other choice left than to settle in a much more open area than the early breeding males.

3-4. Nesting success in relation to water level

The water depth in the study area varied between 45 cm and 152 cm (Fig. 4). Occasionally in late May and June, the Tsuya River was flooded after long-lasting (3 days at most) heavy rains. For the three-spined stickleback this meant that their eggs and fry were in

danger of being washed away. The number of nests washed away by rising water levels was fairly low (3/189 = 1.6 % of the total number of nests; 3/80 = 3.8 % of the unsuccessful nests). In all cases these nests belonged to males which had settled and built relatively late in the season. In the enclosure pool as well as in the whole inlet, nesting success and water level change showed no negative correlation (Table 2). It can be concluded that the influence of changes in the water level on nesting success was not very important.

3-5. Body size and nesting success

Males which first started nest-building after mid May were larger in the average of body length than early nesting males (Table 3). These males had been floaters without a territory during the early period. The early breeders hardly grew after nest-building, in contrast with the late ones which grew further in the breeding season. It was likely that the late breeders had more food available during the floater stage than the early breeders during the same period.

Body size had a slight positive relation to the date of nest building initiation (Fig. 10). In March (the starting month of the breeding season), the body length of males varied from 49.5 mm to 57.5 mm. The main breeding males were 50 to 54 mm in body length. After late April, there were no males that began nesting in body length of less than 51 mm.

There was no relationship between body size and the fate of nesting (hatching success or not) (Table 4). Body size was not significantly correlated with the rate of nesting success.

Also, concerning males that had more than two successful nests, body size was not correlated with the rate of successful nests (Table 5).

Those males tended to be rather smaller (52.8±2.4 mm) compared with the mean size of males, though males less than 49 mm in body length did not breed twice.

3-6. Sex ratio

The operative sex ratio (nesting males : gravid females) was 1: 2.2 (N=10) on late February, 1 : 0.7 (N=32) at mid March, 1: 0.8 (N=102) at mid April, 1 : 0.9 (N=97) at late April, 1: 1.2 (N=84) at mid May and 1 : 1.9 (N=20) at mid June. It is interesting to note that the operational sex ratio early in the breeding period was female-biased, though there were more nuptial coloured males present than gravid females (male : female = 1: 0.7). These sex ratios were significantly different from a ratio of 1 : 1 (X^2-test, $p<0.05$), and changed with time.

3-7. Change of female behaviours

Until the breeding peak, it was often observed that a female did not follow a courting male and did not enter the nest when she reached the nest enterance. Furthermore, even when a female entered the nest a willing female (Kynard, 1978) that settled early (before May 8 for convenience) in the territory came out more frequently without spawning than female in the later period (Table 6). During late May there was a sharp increase of the proportion of ripe females that reacted to courting males and declined again during early June.

Late in the season, several swollen females approached and followed nesting males even in the absence of courtship. Besides, some of them attempted to follow a male without the nest or a non-colored male (Table 6). These females that were eager to mate appeared frequently

since the beginning of May, and there was also a small peak of these females in late May. I observed that in the late period two or three females followed simultaneously the same nesting males almost every day (Fig. 11). These males mostly ignored the courting females without showing aggression.

In Figure 12 swimming routes of gravid females were shown during 10 minutes observation periods at four dates. Early in the breeding period females tended to swim irregularly over the whole pool. There was a significant difference in moving distance between these two groups of females. When the moved distance is expressed as the number of pile sections, the early ripe females swam 4.8+1.8 (mean+s.d.) sections, while the late ripe females swam 3.2+1.4 sections (Mann-Whitney's U-test, $p<0.05$). In late May there was a tendency for females to cluster, whether ripe or not, mainly in three areas: between piles 0-2, 3-5 and 7-9. In pile section 0-2, the cluster consisted of more than 30 females and 5 non-territorial males (Fig. 12).

At a given time, females which had become ripe for the first time early in the season visited territories of nesting males more frequent than females which had become ripe for the first time late in the season (6.2+4.5 visits, N=31, and 2.1+2.8 visits, N=17, respectively, during 10-minute observation periods; Mann-Whitney's U-test, $p<0.05$).

3-8. Inter-spawning interval

The inter-spawning interval ranged between 16 and 28 days for 12 females. The twelve females spawned 1.8+0.6 times of mean+s.d. (range from 1 to 3). An additional study inidicated that two females spawned five times in maximum with a mean of 11.8 days (7 to 21 days in a

range (Mori, in preparation).

4. Spatio-temporal nesting activities of individual male

Reproductive success should be determined by the number of offsping reaching sexual maturity in principle. Although it is very difficult or even impossible for the present to measure a real reproductive success, observations were confined to the fate of progeny in the breeding season, as fry could not be followed after fledging. I analyzed in this chapter when nest-building should be initiated and where a nest should be built in order to maximize reproductive success. The aim of this chapter is systematically to demonstrate general effects of temporal and spatial patterns in nest distribution on an individual reproductive success.

4-1. Nuptial coloration

The intensity of nuptial color often changed with each nesting stage, as already described by van Iersel (1953), and this was especially evident early in the season. Changeable color score of male for each nesting activity was as follows:

a1) Males which nested all with the same color score : 31 males

a2) Males which nested with the different color score : 29 males

a2-1) for same color score between successive nests : 17 cases

a2-2) for different color score between those : 35 cases

a2-2-1) increased score : 30 cases

a2-2-2) decreased score : 5 cases

However, the judgement (Kendall's rank correlation test) indicated

that there was close agreement among nest stages in assigning color scores to each of the males. Therefore, color condition remained relatively stable during the study period.

4-2. Effects of nuptial coloration

The majority of early breeding males were brighter coloured than the late breeders (Table 7). The relative number of bright red males (scores IV and V) was highest during the first three weeks of April in which males could get the high rate of successful nests. Nuptial coloration in males was not correlated with the fate (hatching or not) of their eggs ($X2 = 16.56$, $N= 189$, $df=3$, $p>0.1$). Over the whole breeding period there were always some dull colored (score III) males (Table 7), indicating that some of them were sneakers. Both males which started to build their first nest late in the season and males which repeated nest-building had a duller coloration. Although score of VI generally represented an ending condition of breeding activity, a few males that exhibited the score built the first nests late in the season. Brightly colored males tended to court and attack other fish more vigorously than duller colored males did (Spearman's rank correlation coefficient, $r_s = 0.461$, $p<0.05$; Mori, in preparation).

There was little correlation between gonad weigth, kidney weight and nuptial coloration scores ($R = 0.128$, the quantification theory of multivariate analysis: Hayashi, 1954; Tanaka and Wakimoto, 1983).

4-3. Breeding activity

a) Temporal change in the number of nests

The frequency of nests building showed a distinct bimodal pattern: a large peak in middle April and a small peak in late May. The latter

peak was due to the increase of first, fourth and fifth nesting attempts of each male in late May (Fig. 13). The first large peak consisted of first, second and third nesting attempts, which outnumbered those in any other period. About 70 % of the males which had established territories early in the season (till late April) stopped maintaining them in mid May.

Figure 14 shows the temporal fluctuations in the number of newly-built first nests and the number of newly hatching nests per 5 days. Peak of nesting actively occurred in middle April and early and middle May. Accordingly, the number of nests with newly hatching young showed two peaks in late April and late May.

The number of new-built nests and the number of new-hatching nests per 10 day during the breeding period were given as proportions of the total number of nests (a large peak at early April) and the number of newly-built nests (a peak at mid May), respectively (Fig. 15).

b) First egg-laying date

Increased rate of nests that were firstly laid eggs during 5 days sharply increased in early to mid April and mid May just before each of two peaks of nesting number (Fig. 16). In the last 5-day April, as the increased rate of newly nests declined especially, the cumulative value of newly building nests per 5-day agreed approximately with that of nest laid first eggs.

c) Nesting activities for each male

The numbers of nests and successful nests per male were shown in Table 8. All males established a territory and built at least a nest. Fifteen out of the 99 males never succeeded in hatching young but

built the nest. Of these unsuccessful males, fourteen territorial males built only one nest which remained unsuccessful. One male which built two nests successively also produced no progeny. Seven of these 15 males did not succeed in collecting eggs and the five males failed to hatch fry in spite of collecting eggs. It was unclear that whether the remaining three males collected eggs or not.

About 40 % of all males completed only one nesting cycle. One male built 5 nests and he succeeded twice in hatching. Only one of males who nested three times had three successful nests. Males who built one or two nests had a rate of more than 60 % successful nests. While, males who built nests more than two times succeeded in less than 50 % of them.

The proportion of males with at least one successful nest was considerably lower among males with one breeding cycle (65.7 %) than among males with more breeding cycles (97.2 %, 100 %, 100 % and 100 % for males with two, three, four and five breeding cycles, respectively). Almost all males with two or more nests succeeded at least once in hatching young.

4-4. Nest- and territory-holding periods

Over 70 % of the males nested more than once within the same territory. During a certain period, a male held only territory without the nest. Accordingly, there were some days between territory establishment and first nest-building initiation, and interval days between successive nests. Therefore, the nesting period differed from the territory-holding period. The average territory-holding period was 35+15 (mean+s.d.) days for a breeding cycle. The males nested for 25+12 (mean+s.d., N=60), 48+21 (N=23), and 83 (N=1) days for one, two

and three successful nests, respectively.

4-5. Temporal aspects of nesting activity

a) Breeding attempts

The number of successful nests at a given date was uncorrelated with the number of nests (Table 9), indicating that increased nesting times did not always lead to a higher reproductive success. The probability that a male made another nesting attempt declined sharply in late April after the breeding peak in mid April and toward the end of the season.

Figure 17 indicated how many nests a male built after the first date of nest-building initiation. The number of nests per male clearly decreased with a later starting date. It was interesting to note that the frequency of nesting attempts slightly increased again until late May and declined slowly thereafter. This was due to renesting males and new males that first started building a nest (Fig. 17).

The number of nests was 1.4+0.6 (mean+s.d.) for early starters (till April 20) and 0.8+0.5 for males started first nest-building late in the season. In spite of a similar body size at the early breeding season, a time lag of about 90 days was recognized between the earliest date of first nesting initiation (March 10) and the last date (June 10).

b) Nesting period

The date of initiation of nest-building was significantly negatively correlated with the duration of the successful nesting days (Fig. 18, Table 10). When the first nesting period had a duration of 30 days or more, the second and third nesting period lasted much shorter. On the

other hand, when the first period lasted less than 30 days, the following period was of similar or even longer duration. A successful nesting period lasted on an average about 25 days and ranged from as many as 52 days (Male 39) to 11 days (Male 19). The latter nest (11 nesting days) probably contained raided (stolen) eggs, because the period of 10 days were needed for hatching and 3-4 days for caring for fry (Appendix III).

The large variation in the duration of breeding period must be ascribed to variation in the duration of the nest-building and unmated phase. Even if males started nest-building at the same date, a variation in hatching dates occurred because of an asynchronism in egg-laying. The length of the nesting period was indeed positively correlated with the duration of the nest-building and unmated period and also with the larva caring period (Fig. 19, Table 10). Similarly, for the unsuccessful nests, the duration of the nesting period decreased significantly with the starting date of nest-building ($r = -0.596$, $N=13$, $p<0.05$; mean+s.d. of duration of nesting period = 12.7+8.3, mean+s.d. of date of nest-building initiation from March 10 as day 1= 41.1+13.3).

First egg date was significantly correlated with date of nest-building initiation ($p<0.01$). The unmated duration as well as the nesting duration decreased significantly with advancing nest building date.

c) Actual nest days and reproductive success

The AND was about 15 days; males nesting once: 15.6+2.6 (mean+s.d), N=23); twice: 14.7+3.5, N=36; three times: 15.6+2.1, N=15; four and five times: 15.7+0.7, N=5. Table 11 showed that total nesting days

(TND) significantly correlated with AND, AND/TND (called actual nesting proportion, ANP) and date of nest-building initiation.

Therefore, males which nested only once utilized the breeding cycle more effectively ANP, shorter pre-mating phase, the rate of hatching success and RS-index per male was the lowest. While, males that experienced to two nests spent about half of the all breeding days in order to care the nest without eggs. They nearly always succeeded in hatching fry in at least one of two nesting activities (see Table 8). However, males which built nests more than three times prolonged much pre-egg laying phase and brought about a lower rate of successful nest, so that they seemed to be not able to efficiently have a certain reproductive success.

4-6. Date of nest-building initiation and reproductive success

Table 12 showed that there was not a significant temporal change in the rate of successful nesting. The rate of nesting success did not significantly differ between the early peak breeders and the late breeders (Kendall's rank correlation, = 0.224, $p>0.1$).

While, there was a negative correlation between RS-index per nest and date of nest-builing initiation (Kendall's rank correlation, =0.671, $p<0.01$; Fig. 20). Generally, although the males that nested too early in the breeding season tended to have less RS-index, there was a significant trend that early nesting males had a greater number of successful nests than late nesting ones. Therefore, the late breeders, in comparison with earlier ones, had a relatively low reproductive success (RS-index).

4-7. Nest site location

a) Distance from the shore

The first location of nest site for each individual are drawn in Appendix IV. The nest site location was divided into three categories according to the distance from the shore (Fig. 21): near-shore (distance < 10 cm from the shore), intermediate (10-30 cm) and offshore (30-50 cm from the shore). The near-shore area was most frequently chosen as nest site location. The males initiated first breeding activities in this area, which occupied about 60 % of the total nests. The number of nests built the near-shore decreased up to mid April and thereafter slowly increased, because the nesting space such as a shoreline gradually became a limiting resource. The relative proportion of nests located in offshore varied greatly throughout the breeding season, while the relative proportion of nests at the intermediate position remained relatively unchanged.

b) Cover around the nest

There was no significant correlation between the degree of nest cover and nesting success (Table 13). The proportion of nests built in the 0 % and the 100 % cover categories (Plate III and IV) were much smaller than the proportion in the other categories.

c) Correlation among variables of nest site location

Correlations between variables associated with nest site location were shown in Table 14. There were four significant negative correlations: between nest density and distance to the nearest nest, between nest density and territory size, between distance from the shore and cover proportion, and between cover proportion and territory

size, and one positive correlation.

4-8. Nest site location and nesting success

Discriminate analysis by Mahalanobis' generalized distance (Tanaka and Wakimoto, 1983) revealed non-significant distances between successful and unsuccessful nests on the basis of four nest site variables: nest density within a 50 cm radius of the nest, distance to the nearest nest, distance from the shore and a cover percentage (Fig. 22). Also, the fate of nest was not significantly correlated with the nest density in a 100 cm radius of the nest.

4-9. Nest dispersion and nesting success

The distance to the nearest nest was presented in the frequency distribution in Table 15. The mean inter-nest distance was 37.6 cm for successful nests and 35.8 cm for unsuccessful ones and was therefore not an important determinant of nesting success. The distribution of nests at the peak (late April) is shown in Fig. 5.

Neighbouring males could easily see each other except for males nesting in areas which were completely covered by vegetation. The smallest internest distance was 8 cm for nests in thick vegetation. The maximum internest distance was 126 cm in the case of Male 91 and Male 155 which nested in a relatively open area.

Nesting success was independent of nest density (Table 16). Multiple regression analysis showed that there were significant relationships between the nest densities within a 50 cm (X1) and 100 cm (X2) radius of the nest and the distance to the nearest nest (Y) for successful ($Y=-0.925\hat{N}X1-1.55\hat{N}X2+48.36$, $r=-0.503$, $df=91$) and unsuccessful nests ($Y=-4.73\hat{N}X1-1.13\hat{N}X2+59.65$, $r=-0.461$, $df=61$).

4-10. Temporal change in nest distribution

Figure 23 gave the results of analysis of nest distribution by I method (Morisita, 1959). The index of dispersion, I , has a value of 1 for a random distribution, below 1 for an uniform distribution and above 1 for a completely clumped distribution. The distribution of the nest was significantly different from random. The nests showed an uniform distribution in the peak period (April) and a clumped distribution in the early (mid March) and in the late (late May) periods. Also at the early period, a large number of nests were aggregated in the upper area of the pool, and in the peak period the nests were uniformly distributed in the whole pool.

4-11. Territory size and nesting success

Territory size of successful nest was rather smaller than that of unsuccessful nests, except for nests located in open areas (Fig. 24). The size of territorial area was not related to male quality such as a body size and degree of nuptial coloration (Pearson correlation coefficient using the log(x+1) transformation: $p>0.1$).

Territory size was significantly smaller at high nest densities but this must likely be ascribed to the negative correlation between territory size and the degree of vegetation cover (also see Table 13).

There was no relationship between the duration of the nesting period and territory size (Fig. 25). The duration of the nesting period was significantly shorter when nests were concealed in the vegetation (22+4.4 days for successful nests in close areas; 27.3+5.2 days in open areas; 26.6+6.7 days for intermediate areas). This must be attributed to a shorter fry caring period (open area: 6.6+2.4 days,

N=26; intermediate area: 5.9+2.7, N=67; close area: 4.7+1.8, N=16).

Thus, the size of territory was not an important determinant of nesting success. Further, territory size had no effect on lifetime reproductive success per male.

4-12. Successive nests per male

The present nesting success of a male was independent of the nesting success of his previous nest (Table 17), for except males who built once nest.

Nesting success was also uncorrelated with the number of previous nests (Table 18), suggesting an effect of nesting experience.

There was a significant correlation between date of nest-building initiation (DNI) of the previous nest and that of the later nests, regardless of the nest density. The slope of linear regression line on the relationship of DNI between two consecutive successful nests (S-S) was different from the slopes of the other three cases: S-U, U-S and U-U (ANOVA, $p<0.05$). When the previous nest was unsuccessful, the male began to build the later sooner than when the previous nest was successful.

4-13. Internest construction interval

The male often kept on nesting for about 2 to 5 days after the f completely fledged from the nest (Table 19). The length of the fledging period was probably an indication of the male's persistence to his nest, suggesting that this length was significantly shorter after two previous successful nests than after one previous successful nest.

The number of interval days between successive nests (internest

construction interval) was related to the duration of the breeding cycle of the former nest. The shorter previous nesting cycle the sooner new nest-building was started (i. e., less interval days) (Fig. 26). While, when the previous nest was unsuccessful, the reverse was true.

4-14. Movement of successive nests

As an example, the shifts of nest site location of successive nests per male in the section between pile No. 3-4 was shown in Fig. 27. Repeated nests were classified into four categories on the basis of the moving pattern of the nest site location: at the same site, within the previous territory, to the neighbouring area of the previous territory and far away from the previous territory.

The mean distance away from the initial nest was 50.5+104.5 cm (range: 0 cm to 612 cm, N=79) for all repeated nests, and 78.2+121.8 cm (6 cm to 612 cm, N=51) for renesting in a different site. Twenty eight nests were built at the same site, and 28 males renested within the previous territory. Eight males renested in the neighbouring area, and 15 males far away from the previous nest. Males which nested along the shore clearly tended to stay within or around the original territory.

When the former nest was unsuccessful, the male had a trend to move for renesting (Table 20). More than three quarters of repeated nests were located within the same territory (distance: 0, <30 cm) where the previous nest was successful. If the shift of nest site location after an unsuccessful nest was within the same territory, the new nests were successful in about half of the cases. A large proportion of next nestings in a neighbouring area were unsuccessful when the

previous nest had also been unsuccessful.

The distance moved was significantly longer for males which had a previous unsuccessful nest (63.4+98.2 cm, N=43) than for males which had a previous successful nest (48.3+114.3 cm, N=35) (Mann-Whitney U-test, $p<0.05$). There existed no correlation between distance moved and interval days between successive nests: neither for successful previous nests ($r=0.277$, N=36) nor for unsuccessful ones ($r=-0.003$, N=43).

4-15. Ideal nesting pattern and costs

A successful nesting cycle required at least 15 days: 1-2 days for completing a nest, 10 days for after fertilization till hatching and 3-5 days for caring for fry. If a male started to breed early (March 10 as the first day), and every 15 days thereafter repeatedly started a new breeding cycle without intervals, ideally he could complete seven breeding cycles throughout the breeding season (March 10 to June 30) (Fig. 28). However, in reality the internest construction interval was 26 days on an average. Accordingly, there four breeding cycles might be probably the maximum number that the male could ever achieve during the season, but I have never observed any males who successfully nested four times.

Only Male 68 had three successful nests, and his nesting pattern paralleled the above-mentioned one. However, after the successful third breeding cycle, the male remarkably turned upward as if he would never resume breeding activity and then died in a few days. Although Male 39 initiated nest-building at the earliest date, he completed only two breeding cycles with being successful. After his first successful breeding cycle he maintained the old nest for a long time

and he did not start to build a new nest before mid May. Male 10 started to build his first nest during the early breeding season. This nest was unsuccessful, but his second nest was successful. After that he had two unsuccessful nests. Male 81 built to nest five times, which was the maximal number achieved during the breeding season, but only had two successful nests: the first and the fifth nest.

All males that nested plural times had begun breeding early in the season (Fig. 17). Therefore, the sooner a male initiated nest-building, the more opportunities he could have to complete the breeding cycles.

4-16. Surplus males

Some non-territorial males were present on the breeding ground at a given day during the breeding season. However, it is difficult to determine whether (territorial) space was a limited resource or not by the presence of surplus males. Moreover, surplus males might have had a territory before or will be able to establish one in the future. Anyhow, all males observed in this study have nested at least once, and 85 % of them succeeded in hatching fry. Thus, there were no surplus males present in the study area when the entire breeding season was taken into account.

5. Effects of nest site location on reproductive success

5-1. Classification of nest site type

Six nest-site types were classified on the basis of the distance from the shore and the proportion of vegetated cover in the

neighbourhood of the nest (Table 21). Since territories which were located in intermediate area (see Fig. 21) were mostly in contact with the shore, nest-site types were divided by along the shore or detached concerning the distance from the shore.

Nest-site type A is the most open nest site where any vegetation and/or obstacles to shelter were absent, whereas Type F was covered with algae except above the nest, although two nests were completely covered up by algae even above them (Fig. 29). Thus, nest-site type A and B can be regarded as nests in open areas, nest-site type C and D in areas within the intermediate degree of cover (Plate III) and nest-site type E and F in areas with much vegetation cover (Plate IV).

5-2. Differences of reproduction among nest-site types

a) Body size, nuptial coloration and nest-site type

There was no correlation between body size at the start of nest-building and nest-site type (mean+s.d. for body length of breeding starters at nest-site type A = 52+1.8 mm, N=16; type B = 52.4+2.3 mm, N=20; type C = 52.3+1.3 mm, N=15; type D = 52.9+2.8 mm, N=35; type E = 53.9+1.2 mm, N=6; type F = 52.6+1.3 mm, N=7; Kruskal-Wallis method, $p>0.1$).

There were significant differences in the brightness of nuptial coloration at the onset of nesting among males at each different nest-site type. Males with nest-site types C and D were brighter than those with nest-site types A and B (Table 22). The large number of males with coloration score V had nest-site type D, which probably indicated that most of them started nest-building in the early breeding season.

b) Nesting success and nest-site type

There was a significant difference among nest-site types for the relationship between the number of nests per male and the rate of successful nests (Table 23). Nests built in dense vegetation along the shore (type F) were obviously the most successful ones. The successful rate of nests increased from type A to type F according to the proportion of cover (Kendall test, $p<0.001$). This probably means that nest concealment had a positive effect on nesting success.

For the nests which males first built, the rate of nesting success was 16.7 % at nest-site type A (N=12) and 35.7 % at type B (N=14), 41.7 % at type C (N=12), 50 % at type D (N=50), 71.4 % at type E (N=7), and 100 % at type F (N=7). The successful rate of the second nest in nest-site type A, B, C, D, E and F was 33.3 %, 70 %, 55.6 %, 71.0 %, 100 % and 100 %, respectively. At each nest-site type, the second nest for a male was significantly higher in the successful rate of nests than the first nest.

c) Temporal change in the frequency of nest-site types

Nest-site D was occupied most frequently to build a nest throughout the breeding period (Fig. 30). The proportion of nests built in nest-site type D were more than 70 % of all, and decreased till mid May and then increased. This indicated that the early breeders built their first nest in nest-site type D, and not in type F which had the highest success rate.

d) RS-index and nest-site types

The caring period for fry was significantly correlated with the RS-index, as a measurement of reproductive success per nest, except for a

case of nest-site type A, and both variables differed among nest-site types (Table 24). The caring period of nests in open areas tended to extend relatively longer. Nests built in type D had the highest RS-index, while in type E reproductive success was lowest. Moreover, males clumped to nest obtained a higher RS-index than solitary ones (see below).

e) Nesting period and territory size at each nest-site type

Table 25 showed the comparisons of the nesting period and territory size at each nest-site type, split up into successful and unsuccessful nests. It was obvious that the nesting period of successful nests was clearly longer than that of unsuccessful nests. The mean duration of successful nests was 26 days and was not significantly different among nest-site types.

Territory size of successful nests in nest-site type B were twice larger than the size of successful nests in nest-site type E or F (Table 25). Those males which succeeded in hatching clutches in nest-site type C, D and E had smaller territories than the corresponding unsuccessful males (Mann-Whitney's U-test, $p<0.01$). On the other hand, males that succeeded in nesting in nest-site type A and B occupied larger territories than the unsuccessful ones, but the difference was not significant in nest-site type A ($p>0.1$).

f) Renesting and nest-site types

Males which had built a nest in nest-site type D, A and C tended to select the same type (at unmoving nest or territory) for the next nest. When the previous nest was nest-site type D, most of the later nest (87.8 %) was built in the same nest-site type regardless of the

success or not (Table 26). The rate of renesting in the same type was passably low in nest-site type F (20 %) where the successful rate of nest was the highest. These data suggested that nest-site type F, where the type gave the highest rate of hatching success, was less advantageous for a nesting male than the other sites.

Males which built the nest in close areas tended to renest in the same nest-site type again (Table 27). Almost males which previously nested in nest site type C and D renested in the same site regardless of the fate of the former nest.

The distance moved between successive nests did not significantly differ at each nest-site type in case of renesting at the different nest-site type from the former nest (Table 28). There was a tendency that the new nest was built further away from the previous nest in the open area (type A) than from that in nest-site type D, E and F.

g) Change of territory size between successive nests

When a male renested in a different nest-site type, the territory size changed relatively more (Fig. 31). While, a case of renesting in the same nest-site type, the territory size was relatively constant over the breeding period of individual. This indicated that nest-site type was one factor that influenced territory size.

The variance in territory size between successive nests was significantly lower for nestings in the same nest-site type than for nestings in different nest-site types (Mann-Whinmey's U-test, $p<0.01$), suggesting that movement to different nest site occurred an unstable situation for territorial defence. The mean of coefficient of variance, C. V., for the territory size change is 12.8 % for type A (N=4), 16.8 % for type B (N=4), 14.2 % for type C (N=7), 14.3 % for

type D (N=21), 10 % for type E (N=2), 14.2 % for the total for the same nest-site type (N=38) and 29.2 % for the different nest-site types (N=15).

h) Behavioural differences between nest-site types

The frequency of aggressive behaviours was the highest in the open nest-sites since the nests had to be defended on all sides ($p<0.05$). The frequency was the lowest in the densely vegetated areas in which the nests were invisible (Table 29). The frequency of intrusions was the highest in territories in open areas.

Female visits were significantly less frequent in type E and F than in type A and B. Areas with dense vegetation were suitable for nesting because as already mentioned, the males who nested in those areas could have a high rate of successful nest and they required less investment for territory defence. However, there were fewer female visits in vegetated areas, and so this resulted in a low RS-inde Furthermore, nests in areas with dense vegetation (type E and F) often suffered from raiding males (e. g., sneaker, egg stealer, egg eater) (Table 30). When the raiders approached closely and indeed reached the nest, the owners could first detect and chase them away.

5-3. Reproductive effort of male

a) Measurement of reproductive effort

The reproductive effort (RE) of females is usally measured the weight (energy content) of eggs as a proportion of the weight of food consumed (Wootton, 1976; Wootton and Evans, 1976). Similarly, a male's RE should be measured as the ratio of testis weight to body weight (Pianka, 1978). However, the RE of a male can be also said to

be mainly attributable to behavioural investment, which may be expressed in terms of expenditure of time for defending an exclusive area rather than in terms of weight (energy content). Moreover, gonad weight was totally uncorrelated with male condition: expressed in terms of the weight and length of body and nuptial coloration, but the weight of individual male temporally changed during the breeding season (Mori, in preparation).

In this study, reproductive effort of a male per nest was measured as: RE $= \sum_{i=1}^{5} Xi \cdot Yi$ (i = 1...5), Xi = nesting days of each nest stage (i), Yi = territory size averaged at each nest stage (i). The nesting process was divided into five stages (see the method). Territory size reflected that how much energy a nesting male invested in order to succeed in hatching because there was a significant correlation between territory size (cm^2) and swimming distance (cm) in a given time (r=0.763, N=96, p<0.001). Besides, territory size (log transformed) was correlated with the frequencies of intrusion into territory (r=0.525, p<0.01), threatening (r=0.352, p<0.05) and attacking (r=0.302, p<0.05) against intruders by the territory holder (Mori, in preparation).

b) Effect of nest-site types on reproductive effort

The RE decreased in order of close vegetated area, intermediate area and open area. The RE of nesting in areas with much cover was about 2.5 times smaller than that of males nesting in open areas (Table 25). The RE varied with nest-site type being particularly high in nest-site type A and B (open areas), even when the nest was unsuccessful (ANOVA, p<0.01).

A decline of RE per nest over time occurred in nest-site type B and

D. The RE per nest in both type B (r=-0.7034, N=11) and D (r=-0.5463, N=49) was negatively correlated with the date of nest-building initiation. While no significant temporal trends were found in the other types. The period caring for fry of nests built in type A and B was longer, though not significantly, than that for nests in type C, D, E and F irrespective of the initiation date of nest-building, although later in the season the caring period for fry became shorter.

There was a negative correlation between individual RE (the total RE per male) and date of nest-building initiation (D. N. I.) (p<0.01, Fig. 32). While, for males as well who once succeeded to hacthing a decline of RE was significantly recognized as time passed (p<0.05).

There was a significant correlation between individual log RE and RS-index (r=0.711, p<0.001; mean±s.d.: log RE=3.18±0.34; RS-index=4.3+2.1). Also for the unsuccessful males (log RE = 2.78, N=15), there was a negative correlation between D. N. I. and RE (r=-0.471, p<0.05).

The efficiency of individual RE (RS-index / log RE) was the highest for the male that successfully nested three times in nest-site type D. Males with one or two successful nests tended to have a higher RE efficiency when they nested in nest-site type D than when they nested in the other types (Table 31).

6. Social interaction

I investigated the following issues: a) what a nesting pattern leads to associative nesting linkage, b) how much influences nesting linkage has on reproductive success and also c) what do raiding males and what are ecological significances for them ?

6-1. Neighbouring nesting linkage and breeding synchronization

Neighbouring nesting linkage meant the simultaneous presence of several nesting males which carried directly on territorial interactions each other within a particular area. The number of synchronized nesting days was based on the entire breeding period of a male, that is to say, the period from the date of nest-building initiation of the first nest until the end of the last nesting period for a male. The rate of synchronization (SR) was calculated as:

$$SR = \frac{\text{synchronized nesting days between the two males}}{\text{total nesting days of the two males}} \times 100\ \%$$

"Link nesting" was defined by a SR value of 75 % or more, referring to the cluster analysis (Tanaka and Wakimoto, 1983), among two or more males (Fig. 33; Appendix V). When there was little direct contact between the males which nested in densely vegetated areas, this was not called link nesting even if SR values exceeded 75 %. Link nesting was thus determined both by the presence of a direct behavioural contact and a highly synchronized nesting pattern.

6-2. Structure of neighbouring nesting linkage

The mean degree of synchronization (SR) and nesting activities within each linkage was shown in Table 32. The SR (mean±s.d.) was 84.1±12.1 % for link nesting males, and 46.3±28.6 % for non-link nesting males. There were 5 links consisting of 2 males, 2 links of 3 males, one link of 4 males, one group of 5 males and one group of 9 males. The number of link nesting members was not correlated with the number of successful nests per male and the rate of successful nest.

Clearly, the nesting link mainly occupied along the shore (22/33,

66.7 %) and surroudings with a little vegetation (Fig. 33). The nesting activity tended to be markedly synchronized for nests which were maintained for a long period, but not for short-lived nests. Males consisting of a linkage kept on nesting for 49.2±15.3 days (mean±s.d., N=33), and non-link nesting males maintained a nest for 33.5±12.3 days. There was a significant difference between the two nesting situations (Mann-Whitney's U-test, $p<0.01$). In other words, the members tended to occupy the same site during the whole breeding season, which led to a stable aggregation in which the aggression level among neighbours wanned with time.

Males which would be involved in a nesting link initiated to nest at an earlier date than non-link nesting males: date of first nest-building initiation was 27±17 days (mean±s.d.) from the date of March 10 for the link nesting males and 38+20 days for the non-link nesting males (Mann-Whitney's test, $p<0.01$).

6-3. Presence of a neutral zone

Between two nesting males, a neutral zone, that is to say, a certain space between two established territories in which the owners hardly intruded each other has often been recognized (Fig. 34). Neutral zone was only formed in a nesting situation in which the nests were built early in the breeding season. While, this zone was not observed between nests that were built late in the breeding season and/or in a non-link nesting situation.

Early in the breeding season (nest-building phase), link nesting males were often more aggressive than non-link nesting males (Fig. 35). At that time, there was no difference in aggressive level between link and non-link nesting males (Kendall's rank correlation,

$p>0.1$). However, the level of aggression of link nesting males wanned with time. In the middle of the nesting cycle (after about 10 days from nest-building) of link nesting males, the frequency of aggression sharply declined, and then this might indicate that a neutral zone was established.

6-4. Territorial defence against intra-linkage males and other males

Figure 35 also shows that for link nesting males there were significant differences in the frequency of aggression against different intruders: neighbouring males that belong to the same link and other males, such as floaters and raiders that did not belong to the linkage. In the early stage, the link nesting males aggressively directed at neighbouring males mostly than at other fish (Mann-Whitney's U-test, $p<0.01$, Fig. 36).

The frequency of aggression against intra-link males gradually clearly decreased with time (Mann-Whitney's U-test, $p<0.001$, Fig. 36), while in comparison with it, that against other males was relatively constant.

6-5. Aggression difference between link and non-link nesting

The frequency of aggression was significantly lower in link nesting males (3.3 ± 1.8 times per 5 min., N = 66 cases) than in non-link nesting males (5.3 ± 2.7 times per 5 min., N = 66 cases) including all nest-site types, except the early period (empty nesting stage) of the nesting cycle per male (Mann-Whitney's U-test, $p<0.01$). When the statistical analysis of frequency was also separately calculated only regarding nest-site type D, there was a significant difference in the aggressive behaviours between link nesting males (2.3 ± 1.8 times) and

non-link nesting males (4.2 ± 2.1 times)(Mann-Whitney's U-test, $p<0.01$).

6-6. Temporal change in aggression level

The level of aggression of non-link nesting males against intruders, irrespective of neighbours or others, did not change with time (Fig. 36; Spearman's rank correlation, $p>0.3$). While, within most links, the nesting males had the highest aggression level at or just after nest-building period (empty nesting stage), and then the level smoothly decreased during the nesting cycle of each male (Figs. 35 and 37). However, until seven to 10 days after nest-building initiation, the proportion of time spent in aggression of link nesting males was significantly higher than that of non-link nesting males (Mann-Whitney's U-test, $p<0.01$). After approximately the first 10 days during the first breeding cycle, the aggressive interactions were stabilized with a low level suggesting the establishment of a neutral zone.

Later (stage 1: after fledging fry), link nesting males were less aggressive towards neighbouring males within the link to which he belonged. The aggression level against other males and females remained relatively stable during the breeding cycle, though slightly decreased also. However, in the case that a male moved his next nest outside of the previous territory (e. g. Male 81 and 20 in Fig. 35), the aggressive interactions raised again to initial levels. This suggested that there was a high cost to move the nest or to establish a territory elsewhere.

6-7. Description of communal defence against raiders

Figure 38 shows an example of comparisons in territorial defence

against a raiding male in link nesting situation (Fig. 38-A) and in non-link nesting (Fig. 38-B). A raiding male without his nest approached the nesting sites between pile 9-10 where the place was residented by link nesting males, Male 180, 147 and 182. And then, the nest raider tried to intrude into the territory or nest of Male 163. But because the raider was threatened, attacked and chased away by the owner, subsequently he closed to the territory of Male 180. This event may be immediately known over neighbouring males. Male 180 who adopted the head-down threating chased away the raider without any direct attacks. Further, before the raiding male attempted to go towards the nest of Male 147, he fled only by an approach (about 20 cm distance) of the resident Male 147 and therefore could not even close to the territory. Then, the raider turned at once towards Male 182. However, he rapidly went throughout the territory of Male 182, because the resident Male 182 only oriented a few centimeters distance to the opponent. But, the raiding male succeeded in reaching and rushing into the nest of Male 90 (at nest-site type D) though he might not eat eggs and fled away because of attacking by the resident. Therefore, it was suggested that link nesting reduced the accomplishment of nest raiding and predation as a result of communal defence.

On the other hand, in non-link nest situation in close areas, Male 160 and Male 178 severely suffered from a nest raiding (Fig. 38-B). Male 150 consecutively succeeded in intruding into territories of the two non-link nesting males. Afterwards, Male 150 sometimes (five times on that day) went intentionaly towards Male 160 and focused to intrude into the territory. These raiders were furiously attacked and chased by the owner, and scampered away.

Moreover, the raider Male 105 easily reached the nest of Male 150

which loacted in non-link nesting situation of open areas, and draw out the eggs. Then, the eggs attracted other males and females as well as Male 105 and were entirely eaten by them.

Thus, it is satisfactory to consider that the raiding behaviour in link nesting situation tended to occur less successfully than that in non-link nesting and/or in close areas. Nests in densely vegetated areas, which leaded to non-link nesting, indeed often suffered from raiding behaviour.

6-8. Neighbouring link nesting and reproductive success

The number of successful nests per male and the rate of successful nests was strikingly less for a non-link nesting male than for a link nesting male (Table 32).

The linkage size, that is so say, the number of nesting males of which it was composed, was uncorrelated with individual RS-index (Table 33).

Within nest-site type D where the nests were most frequently built, there was clearly a link nesting effect which influenced on reproductive success, hatching rate, the number of successful nests and RS-index per male (Table 34).

The link nesting situations were generally found along the shore. Most of these nests belonged to nest-site type B or D (Table 35). Nest-site type F was also situated along the shore, but in dense vegetation and was therefore excluded from the linkage. Sixty % of all nests were built in type D and about three quarters of these nests were successful.

This chapter shows evident differences that nesting males who clumped synchronously in nest-site type D gained more successful nests

per male, higher rate of successful hatching and higher RS-index compared with males non-linked in other nest-site types.

6-9. Descriptions of nest raiding

a) Raiding behaviour

In this study, an intruder which really reached the objective nest would be used the term of 'raider' according to Wootton (1971, 1972). However, even if an approach was only recorded at the observation time, when the raiding behaviour of the intruder was before witnessed, they were regarded as raider (Male 105 and 4 in Table 36). Male raiders first attempted to approach the nest of another male. In doing so the raider's breeding coloration faded and he sank to the bottom upon reaching the territory border of the other male (Plate V). When very close to the strange nest, he dashed at it. When the raider was detected by the owner before reaching a nest, he was always attacked, frequently with tenacity and then fled.

Nest raiding was observed at 21 nests (or males) and 33 times (Table 36). About 67 % of the raiders nested successfully to breed. While, nests which suffered from raiding had a success rate of 52.4 %, which was not much lower than expected. Thus, a nest raiding had little bearing a hatching success.

In the three-spined stickleback unlike in salmonids, the raiders and the raided males did not significantly differ in body size (52.3±2.1 mm and 53.4±2.9 mm, respectively; Mann Whitney's U-test, $p>0.1$), and the body size of both was not significantly differed from the mean body size of all males ($p>0.2$). Comparative behavioural analysis between the raided males and the raiding males did not provide any dominance relationships between them. The nest raiding was usually

undertaken by territorial males with nesting (30/33, 91 %). The nests of raiders could also be raided and the raided males could become raiders as well (examples of Male 105, 91, 34, 156 and 160 in Table 36). It was unclear, when and how these reversals developed. The raiders did not react aggressively to the nest owner except in one case (Male 104).

The appearance of raiding nest was closely connected with nest site location. The highest proportion was made in nest-site type E and the lowest in type D (see Table 30). As already mentioned in the previous chapter, the group nesting males seldom suffered from the raiding behaviours except for the nests of Male 20 and Male 180 which were raided successfully.

The mean distance (±s.d.) from the raider's nest (or a regular site when the raider had no nest) to the raided nest was 215.9 (±359.3) cm, and ranged from 30 cm (Male 4 to Male 90) to 1395 cm (Male 48 to Male 105). The swimming distance for raiding thus varied greatly irrespective of the nest situation (Fig. 39). In 62.5 % of the cases, nests of neighbours were raided. The frequency of nest-raiding was not correlated with nest density (the number of nests within a radius 50 cm from the nest) (Kendall's rank correlation test, $p>0.2$).

The male whose nest was raided tended to have a brighter nuptial coloration than the average male whose nest was not raided, but this difference was not siginificant (mean±s.d. for coloration score of raided males = 4.4±1.0, that of raiders = 4.0±0.8 and that of non-raided males = 4.4±0.8).

Some females formed aggregately shoals to raid nests. The nest owner situated considerbale difficulty in chasing off such shoals of raiders. Only, on May 29 one female which was not gravid pushed the

nest and rushed away by the owner's attack.

In addition, the nest-raiding behaviour may have depended on weather conditions: almost of nest raidings (84.8 %) occurred in sunny weather condition. There was no correlation between nest raiding and time of the day (from 8:00 to 17:00) although most raidings were observed between 11:00-15:00.

b) Effects of nest raiding

Nest raiding might result from stealing of eggs (bringing eggs which were fertilized by the other male into one's own nest), eating of eggs (Plate VI), destruction of the nest to obtain nest material for one's own nest, and disturbance of courtship (Table 36).

What the raider did after reaching the strange nest depended on the stage of raided nest (Table 37). Most raiders and raided males at the time of raiding appearance were situated in the sexually matured phase. Raiding behaviour occurred at all stages of the nesting cycle, and some raiders had even no nest or territory.

Male seemed to be capable of stealing and eating the other's eggs while being the parental phase. When the raided nest included no eggs, the raider (Male 90, 105 and 143) sometimes tore at the raided nest and thus destroyed it. When the raided male had eggs in the nest, the raider (Male 91, 58, 22, 4, 7 and 134) attempted to steal the eggs either to eat them or to carry the stolen eggs back to his nest. Stealing the other's eggs to bring them back to the nest was performed by some males without eggs in the nest. I observed twice that a raider who had stolen eggs from a resident's nest swam over a distance of several meters (about 5 m) to bring them back to his own nest, but eggs could also be stolen from neighbours (on an occasion of

raiding Male 150's nest by Male 105). Male 91, 58, 22, 4, 7 and 134, when they had no nest, did not steal eggs.

Some raiders dashed into the other's nest just before the nest owner could fertilize the eggs and managed to steal a fertilization . The sneaking behaviour was never observed for non-territorial males. Also, raiders sometimes disturbed the other's courtship by lying across the nest and thus prevented the female from entering it.

There was a trend that some raiders, possibly intentionally tried to raid a particular nest. For example, the raider Male 105 attempted more than once to raid the nest of Male 69, Male 17 to the nest of Male 25 and Male 59 to Male 20's nest. The owner whose nest was raided or was about to be raided rather tenaciously chased after the male, who had experienced to raid the nest before, over a distance of more than 1 m out of the territory.

The seizure of the nest was once observed on the nest of Male 11, which suffered from Male 104 (see Fig. 38-B). Although a head-down posture for a threat was not a characteristic of raiders, Male 104 has often shown the posture with wandering close to the territorial border of Male 11 for a few days. In April 27, Male 104 frequently began intruding into the territory of Male 11, neighbouring male, and on the border they were threatening and attacking each other during several hours. After all, at 15:26 April 28, Male 104 drove away Male 11, and forcibly changed his nest on the position of Male 11's nest. After that, Male 11 located in the neighbour of the previous nest site (around the nest of Male 122) and mainly floated bewteen the pile 7 and 9. Then April 30, he began to build a new nest, which was about 220 cm distance away from the previous nest, in open area within the pile section 7-8. Besides, some observations that Male 156 frequently

intruded into the territory of Male 160 at the end of his breeding cycle might be considered as an attempt to take over his territory. hese two raided nests were situated in dense vegetation where intruders were hard to be found and so raider(s) could easily intrude into the territory and approach to or reach the concealed nest.

Furthermore, several females and/or non-territorial male(s) (shoal) aggregately raided a certain nest. If the nest contained eggs the members of shoal could eat them (Shoal A in Table 36). The shoal of raiders occurred only in the open and intermediate covered areas, but not in densely vegetated areas. The resident male was hardly able to guard his nest and eggs against such a raiding shoal. Although he sometimes poked the females, it was extremely difficult to prevent them from raiding the nest and was very hard to be chased away from the nest and/or the territory.

When a sneaking male succeeded in reaching and raiding a nest, his activity and/or the result of raiding, for example a few eggs dropped from the nest to or his mouth, probably attracted other males and some females. Such a sight of raiding seemed to be a potent situation for eliciting raiding behaviour of the surrounding fish. For instance, it could be thought that the raiding Shoal B was a case elicited by seeing a nest-raiding behaviour of other(s).

As there were some nests of which eggs were often stolen or eaten by a raider and/or raiding shoal, reproductive success (measured as the number of hatched fry) was expected to be significantly higher in non-raided nests than in raided nests, but no significant difference was found (ANOVA, $p>0.3$). It was likely that reproductive success was primarily determined by temporal and spatial aspects of nesting, and the effects of raiding behaviour therefore have been masked.

7. Discussion

7-1. Brief review

Reproductive behaviour of the three-spined stickleback, *G. aculeatus* males has numerously been studied in experimental and field situations since 1930's (ter Pelkwijk and Tinbergen, 1937). Some of these studies have shown that a certain factor is simply related to reproductive success. Attention has particularly been paid to two factors: territory size (Assem, 1967; Black, 1971) and nest concealment (Moodie, 1972; Kynard, 1978; Sargent and Gebler, 1980; FitzGerald, 1983; Whoriskey and FitzGerald, 1985a, 1987). For example, Assem (1967) in a laboratory study found that males with a larger territory had more eggs in their nests than males with a smaller one. Consequently, male competition occurred to obtain territories of superior size (Assem, 1967; Black, 1971). These reports suggested that a male should defend the largest territory possible to maximize its reproductive success. In Canadian populations, however, Sargent and Gebler (1980), Sargent (1982) and FitzGerald (1983) described no relation between territory size an reproductive success (number of eggs per nest). While, Moodie (1972), Kynard (1978) and Sargent and Gebler (1980) found that nests hidden in the vegetation, where they were protected from egg predators, contained more eggs than those built in open areas. Hence, FitzGerald (1983), FitzGerald and Whoriskey (1985) and Whoriskey and FitzGerald (1985a) stated that nest site characters were more important determinants of reproductive success than territory size. The

Canadian researchers found that nest-site characteristics (percentage cover, nest depth and distance from the bank) influenced the number of egg per nest. However, their studies, failed to establish a correlation between measures of male quality, indicated by body size, nuptial color and aggressive behaviours, and reproductive success of a male, and therefore they concluded that nest site topography alone could be an important determinant of the success without recording a lot of traits of individual behaviours. Furthermore, they mentioned that intraspecific competition may not be an important determinant of individual reproductive success, and environmental fluctuations play a principal role in determining egg production patterns.

The above-mentioned studies, however, have not dealt with reproductive success for each male during the whole breeding season and they paid only attention to a few spatial aspects (territory size and concealment) of nesting, but never any attention to temporal ones and social structure. Furthermore, they therefore can not explain the structure of a breeding population in relation to reproductive success during the entire breeding season.

The temporal change in nesting activities which is connected with reproductive success has hardly been individually in detail reported in sticklebacks and possibly almost no in the other nest-building that inhabit freshwater. This study clearly provided that the complicated variations of temporal and spatial breeding activities strikingly influenced on an individual reproductive success.

7-2. Effects of nesting patterns on reproductive success: Mechanisms by which the nesting activities in time and space affect reproductive success

There are few quantitative studies with regard to the ecological significances of temporal and spatial reproductive activities although descriptive and physiological studies concerning the onset of reproduction are common to fish biology (pacific herring, in Hay et al., 1988; brown trout in Bagenal, 1969; platfish in Sohn, 1977; three-spined stickleback in Baggerman, 1957 and Wootton, 1973). In this section, ecological problems to which I would pay some attention are effects of the territory and male qualities, mating system and the timing onset and space utilization of nesting activities on an individual reproductive success: situations of each individual.

The breeding system, which affects individual reproductive success, of male stickleback is schematically summarized in Figure 40. The below discussion should be understood in company with this figure, which is interpreted by Table 38 showing the correlations between reproductive success for each male and a number of breeding factors.

a) Characteristics of territory and male

This study evidently provided that the territory size, female visit and intrusion of other males were easily influenced by territory quality, in the form of nest site characteristics. As the result, life reproductive success occurred a considerable variation between male individuals. It was, therefore, very important to decide where a male built the nest. Male that initates breeding early occupies an optimal nest-site, where the territory adjacent to vegetation along

the shore, for obtaining high reproductive success. This topographic complexity can decrease the frequncy of intrusion, which were thought to provide the efficient territorial defence (Kodric-Brown, 1977), and increase the mating chances because of the oviposition site least vulnerable to disturbance of spawning behaviours by other fish (Jones, 1981; Thresher, 1983). This may also propose shelter sites for the nest owner and hatched fry to avoid predators.

This study also provided that the individuality of the male, morphological characteristics such as the brightness of nuptial color, was associated with the onset of individual breeding and was likely to affect fitness. Mate choice and dominance hierarchy was connected with the brightness of breeding coloration (Rowland, 1982; Bakker and Sevenster, 1983). Bakker and Sevenster (1983) and Bakker (1986) used a method of four-point coloration scale in the three-spined stickleback male and found that the brightness of coloration is an important determinant for males to obtain mating advantages or dominance. While, unlike many other fishes (Perrone, 1978; Downhower and Brown, 1980; Loiselle, 1982; Katano, 1990), in the presented three-spined stickleback, male body size as one of male qualities was neither correlated with hatching success, reproductive success, territory size nor nesting site selection. Thus, male body size was not so important for his reproductive success.

It is often difficult to separate effects of male quality and territory quality on reproductive success because they are usually correlated with each other. The ability of males to hold a territory of superior quality may allow females to evaluate the real quality of males. In short, the breeding initiation early in the breeding season, which might be one aspect of male quality, promoted the

occupation of good sites, so that a driving nature of when and where a male began nesting made a decision for degree of reproductive success.

In case of assessing mate choice in species forming territoriality, we must seriously distinguish between male quality and territory quality. Besides, an importance of this problem was also flexibly affected by how far we can hold perspective of the fact that the territory and male qualities have a variable disposition in space and time. Needless to say, there would also be drastically individual variations in mating and aggressive abilities which correlated with nuptial coloration (Rowland, 1984) and parental one regardless of territory quality. What aspects of male quality and/or territory quality have the largest influence on male reproductive success should be considered in future experimental treatments.

b) Variability in mating system

A mating system includes the frequency of sexual attachments, the kind of pair bonding, and the distribution of effort in parental care, if it exists. So far the concept of mating system, which is subordinate to that of social system, has been vaguely regarded as static entities that remain invariant over time. This viewpoint has allowed us to oversimplicate the mating system as a constant and uniform style such as monogamous, polygamous (polygyny, polyandry and harem) and one-male-grouped (Pianka, 1978). This oversimplication may partly originate in a concept of species-specific behaviour defined by classical ethology (Tinbergen, 1951, 1953; Lorenz, 1965) and/or in ecological viewpoints of population dynamics and community that dealt with a or more species in an arbitrarily setting area (Macfadyen, 1965). Also its derivation might lie in a word itself of

"system" being imagined a closed impression. Anyway, a terminological problem of definition is occurring here and it appears that the classification of mating system has ever been an oversimplication (Turner, 1986). We now should avoid trying at least to construct a complex classification of systems such as a criterion on the base of the number of mates acquired.

Basically, the mating system of the three-spined stickleback is a form of polygamy. The nesting male could collect and fertilize clutches of up to ten, and obtain a few different females at the nest (Mori, 1987b) during a short period (from one to several days). The mating system could be called the resource defense polygyny. On the other hand, it was clear that the female was able to spawn up to three times with different males. There was also an evident that some females spawned only once in their life time. Where females actively choose displaying males early in the season, they were generally exerting mate choice. Therefore, there was a considerable variation in male reproductive success.

The probablity that increase mate aquisition was strongly influenced by the temporal pattern (change of operational sex ratio) of availability of sexually receptive partners (Halliday, 1983; Thornhill, 1986). Consequently, the mating system is neither uniform nor fixed for the species through the whole breeding season (Warner and Hoffman, 1980; Fricke, 1980; Kodric-Brown, 1988), but vary temporally, spatially and individually. It could be also stated an individuality of mating system, which might be called 'individual mating strategies' (Turner, 1984). This variability does not indicate a shift of the mating system which varies through environmental heterogeneity in temporal and spatial distribution of resources within

or between population, for example variance of breeding structure by a density-dependence such as a change from territorial to lek organization with increasing density (Arak, 1983).

c) Individual reproductive success and time utilization

The three-spined stickleback males in the present study neither had a regular breeding cycle as a species or a population nor simultaneously initiated breeding like a Salmon, *Salmo kita*, and an anadormous stickleback, *Gasterosteus aculeatus* forma *trachurus* (Moyle and Cech, 1988; Wootton, 1976, 1990). Further, newly breeding males did not reach the breeding ground one after another and begin nesting in succession. Even if the body length of males attained maturity size at the onset of reproductive period, some males could not build the nests and the body size at starting to nest differed individually between males and others started nesting.

The consequence was that the difference in the date of nest-building initiation caused a variation in the number of nesting times per male. This study suggested that males which started to nesting earliest were able to have the potential ability to breed up to five times in the whole breeding season and indeed the maximum achieved was three times. Similarly, laboratory study also showed that the male could complete up to five breeding cycles (Wootton, 1984). However, increased nesting times did not always lead to a higher reproductive success, because the number of successful nesting times was not positively correlated with the total number of nesting times. While, males which bred twice could certainly gain a stable reproductive success since they were guaranteed at least one successful nest and then could manage to lose relatively a little investment to succeed in nesting.

Although the number of nesting cycles was also strongly controlled each nesting days with a crucial period for hatching during the restricted period of breeding season, the period of nesting days decreased significantly with advancing building date. The causes of decline might be explained as follows:

1) A main reason that early nesting males delayed their first egg-laying date because males generally tend to begin settling in the breeding ground earlier than females. Therefore, there was a rather time lag between date of nest-building initiation and date of first egg-laying.

2) In connection with the above matter, there was a difference in operational sex ratios (Emlen and Oring, 1977; Emlen, 1984) at the breeding site early in the breeding season in comparison with those late in the season (Kynard, 1978; Whoriskey et al., 1986). The sex ratio was male biased early in the season and it was therefore likely that nesting males might have a rather variation in opportunity to mate and faced a severe competition for females (Halliday, 1978).

3) Change of female behaviour related to mate choice (Ward and FitzGerald, 1988). When a female early in the season approached a male but was often ignored, she immediately gave up courting to the male and oriented towards other males, probably because at that time there were many potential males. And then, the breeders of both sexes do not frequently complete courtship and mating. On the contrary, late in the season, gravid females persistently maintained courting for some time. The result may indicate that courting females early in the season could be more choosy as compared to females late in the season (Kynard, 1978). As the season progressed, change of female behaviour may lead to variation in the period of nesting days.

4) Male that nested early might need a considerable time and energy spent to establish a territory and start nesting because of the intense competition for breeding resources (e. g. tree swallow in Stutchbury and Robertson, 1987). Male-male competition might interfer with parental care, for example, so that these males spent more time in aggression and fanning (Assem, 1967; Sargent, 1985; Ward and FitzGerald, 1988). This was suggested by the high level of aggression at each nesting behaviours early in the breeding season.

5) When breeding was advanced, the low intermale competition stably appeared among males that nested early compared to males that started nesting late. As a result, the situation would provide a small number of nesting days since early males could reduce time to defend the territory and aggress the others.

6) Another possible explanation for the prolonged breeding cycle was that the mojority of males had neither nesting nor breeding experience yet early in the season and therefore needs more time to complete a nest and mating. Effects of breeding experience (usually as age in year and previous breeding attempt or not) on reproductive success have been numerously studied in mammals and birds (see Clutton-Brock, 1988). Indeed in the present fish, movement of nest-site was more likely after breeding failure than after success, so that males may properly evaluate their previous nesting event as experience.

It is worthwhile to note that reproductive success per nest as well as the number of nesting days decreases as breeding season progress. In many avian species, clutch size per nest decreased with the progress of breeding season (e.g. Perrins, 1965; Orell and Ojanen, 1983; Cody, 1985; Bancroft, 1986), but the decline has hardly been reported in fish. In most cases of birds, this temporal change in

reproductive success was treated with food distribution in relation to feeding young. However, the temporal pattern of the three-spined stickleback had most relation to the occupation of nest site which might be attributed to differences in the visit of females and intruders. Males that early settle along the shore of the breeding ground could obtain a high reproductive success in spite of a male-biased, implying a shortage of females, early in the season. These males earlier established territories and completed a nest, and then met with more frequent female arrival in the breeding ground and female visit in the territory. Because of a significant increased bias towards females as the season progress, they may have more opportunities to mate, as a result could contain more clutches in their nests that early were built. Thus, this early situation would probably indicate more intense intra-male competition directly not only for the few available females but also for occupying a good nest-site or territory.

As these results, because the early breeding males which efficiently invested time and energy into the two succeessive nesting activities along the shore have fundamentally much more opportunities to increase the number of successful nesting times and to occupy a suitable nest site for breeding, they could gain a high reproductive success. Moreover, there was an advantage to breeding males earlier in the season since young hatching early may be more likely to survive a shown by the avian studies (e. g., Perrins, 1966). On the contrary, the late breeders tended to be forced to nest in open areas with a higher risk of intrusion, nest raiding and a fewer number of females, and therefore have gained a lower reproductive success. This difference also determinately derived from interindividual interaction

with accompanied by forming a synchronous nesting linkage among neighbours.

7-3. Social structure on the base of interindividual interactions

a) Process and functions of neighbouring nesting linkage

Principally, social structure is founded on an intraspecific interaction which consists of both exculsive and attractive behaviours, at least between two individuals. Some animals tend solitarily to distribute their nests over the breeding area. Others assemble to nest within a relatively small part of the breeding area: breeding activity in colony or in group. Those two opposing distributional patterns, which may have both anti-intruder and/or mating functions, have been well documented by the many general reviews (Morisita, 1961; Davies, 1978; Emlen, 1984; Begon et al., 1986).

In the three-spined stickleback, there was a clear tendency of clumped nesting distribution with territoriality. There were no indications that limited habitat availability forces males to collectively nest in colony. In the colony, the males alomst evenly distribute their nests each other by territorial behaviours. Since the fish assembled to breed in some restricted areas in the stream, one might call it colonial nesting pattern, like a term of avian study (Lack, 1968; Snapp, 1976) with some solitary ones, that is, semicolonial breeding. However, the colonial pattern of birds did not necessarily mean the nesting link which was here defined on the basis of direct behavioural interactions between synchronous breeding males during individual breeding period at each breeding site. The linkage

proposed in this study is an aggregating pattern of nests even within a colony. In the colonial area, variations in microsite quality and breeding onset could have effects on the settling decision of individual and thus on the occurrence of nesting linkage.

Neighbouring nesting linkage developed synchronously during the first seven to ten days after nest-building initiation began to be formed mainly along the shore early in the season and then was established till approximately the end of the first successful breeding cycle. The male often occupied the same place during the whole breeding period, which led to a stable breeding aggregation in which the level of aggression among neighbours waned with time (van den Assem, 1967; van den Assem and van der Molen, 1969) probably as a result of habituation ("dear enemy" effect, Peeke, 1969; Peeke et al., 1971). The genetic relatedness might be unnecessary for the formation of nesting linkage. This process was composed of spatio-temporal synchrony of nesting activity and interindividual interaction of behaviour, and it may usually arise to sociality such as a situation of neighbouring nesting link. Long-lasting stable interaction of related individuals can allow the development of many complex aspects of the social system. Therefore, the important point to detect the social structure and function of the animal is a process which formed an interaction throughout the long-term period.

It was possible that neighbouring nesting linkage contributed to 1) a high reproductive success through facilitation of mating frequency as shown the result that link nesting males raised more fry than solitary nesting ones, 2) communal defense of the breeding area by the exchange of information among linkage members about approaching raiders and intruders and 3) the simultaneous attack by several

breeding males consisting of the linkage, in other words, individuals who shared common territory boudaries could jointly attack or threaten intruders (e. g., bluegill in Dominey, 1981), and 4) possibly the breeding synchronization functioning to reduce intrusion. Moreover, the constantly reduced intensity of aggressive level in a link nesting situation allowed the males to devote more time to fanning compared to even males which nested in dense vegetation.

b) Ecological significances of raiding behaviour

This study showed that objects of nest raiding included three categories of stolen fertilizations, egg-stealing and egg cannibalism. These various forms of raiding might be manifestations of inter-male competition (Li and Owings, 1978; Sargent and Gebler, 1980). Since a raiding male was also available to be the victim of sneaking and was not an inferior individual that had neither built a nest nor a hold a territory, there must be a net of benefit and cost in this behaviour. Therefore, it was unlikely that nest raiding by male made up a fixed dominance hierarchy among males.

Sneaking of sticklebacks has been first described in a laboratory study (Assem, 1967) and subsequently globally reported in natural populations (in a small stream of England, Wootton, 1971; in Wapato Lake, Washington State USA, Kynard, 1978; in tidal salt marsh pools Quebec Canada, Whoriskey and FitzGerald, 1985b; in Crystal Lake western Canada, Ridgway and McPhail, 1988b). If sneaking of fertilizations increases the reproductive success of the raider, then this raiding behaviour is probably one of alternative reproductive tactics (Wootton, 1984). In other families of fish, for example in the salmonids, it has been reported that sneaking of fertilization

occurs generally by small male(s) without the nest or the territory (Maekawa, 1983; Gross, 1984). While, the sneaker of stickleback did not show any significant difference in body size from normal male, and later the most normally nested for breeding. This fact indicates the appearance of raiding behaviour is attributed to an alternative surrounding situations at each individual. The genetics underling the alternative reproductive behaviours have not been yet known.

In some species of fish, it has been well known that females prefer to spawn in nests which already contain eggs (the three-spined stickleback in Ridley and Rechten, 1981; the tesselated darter in Constanz, 1985; the river bullhead in Marconato and Bisazza, 1986; the fathead minnow in Unger and Sargent, 1988). The reason why female seeks out a nest with eggs may be explained by a viewpoint that when she firstly spawns in an empty nest, a higher probability may occurred that her eggs will be eaten by raiders or by the nest owner (filial cannibalism). Therefore, males would attempt to contain eggs in any way as fast as possible due to a female preference. As a consequence, egg stealing by male would increase his own reproductive success. Practically, egg stealing mostly appeared in the pre-egg laying stage early in the breeding season, thus it may lead to be efficient in obtaining a higher reproductive success (Mori, unpublished data). However, this study resulted that males that stole eggs never obtained a higher reproductive success compared to normal nesting males. In addition, Whoriskey and FitzGerald (1985a) negatively mentioned that since the raiding male often abandoned his own nest the day after he stole many eggs, he gained little benefit from the stolen eggs themselves.

Egg cannibalism was hard to be explained in term of reproductive

success. Whoriskey and FitzGerald (1985b) concluded that density-dependent cannibalism is important in population regulation of *G. aculeatus*. However, egg cannibalism in the present population seldom possibly affected population abundance since the cannibalism did not occur so much compared with Canadian population. The Canadian researchers reported that females eat more eggs than males, and they speculated on possible reasons for egg eating to be adaptive for females (FitzGerald and Havre, 1987). For example, the females could gain energy for producing their own eggs and sexual tactic to obtain a partner. Similarly, in the present study, female packs were the most important egg eaters. There might be evidence for intense female competition for mates. On the other hand, male benefited to eat other's eggs (heterocannibalism termed by Rohwer, 1978) as an investment in future reproduction. However, there was no evidence that the egg eater increased reproductive success. A discrimination should be made between egg cannibalism by male and that by female since cannibalism may have different function in the two sexes. The fish was unlikely to consume eggs only as a food source, because there were also abundant food supplies in the studied stream (Mori, unpublished data). It still remained obscure how egg cannibalism could be adaptive in ecological significances.

7-4. Future field studies

There are thematic subjects on which I hope to focus my attention How does each male determine where and when he establishes a territory and begins building a nest ? These subjects, for example, inexorably lead to two questions. One is a question concerning sex ratio, which plays an important role in reproductive success. The operational sex

ratio, which fluctuates with time progresses and by micro-locality, was evaluated from the proportion of nesting males and gravid females in the present study. However, to understand a real operational sex ratio, which influences actually the mating situation for individual, only males that are immediately willing to court should be taken into account. The other is, to what I wish to study in natural situations, the timing of reproduction must ensure that the larvae are hatching at a time of year when the size and abundance of the prey are appropriate, and when and where predators are as little as possible.

In a future field study of three-spined sticklebacks, throughout resulting the above mentioned questions, I will concentrate on understanding inter-individual interaction at each microhabitats of individual breeding situation, if possible all through their natural life span (Clutton-Brock, 1988). Some future studies might be needed to individually examine the sociality with special reference to the individuality: genetic polymorphism, ontogenetic characteristics, individual experiences, recognition ability, situation of surroundings and interindividual interaction in a certain group or population over an entire period.

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Summary

1) The breeding success of three-spined stickleback (*Gasterosteus aculeatus* L., forma *leiura*) males in a small stream of the Tsuya River, Gifu Prefecture, Central Japan, was studied with reference to timing of nesting initiation, space utilization for nesting and social interaction. The observations were made almost daily during March to early July 1988 along the shore at a distance of 1-2 m from the fish and nests.

2) All the males in an enclosed study pool were individually marked (99 males). Furthermore, a total of 67 females were marked and observed weekly. The males were individually observed and their agonistic courtship and parental behaviour as well as their reproductive success were quantified.

3) Nest sites were categorized in six types (type A to F) on the base of both the proportion of cover around the nest (3 degrees) and distance from the shore to the nest (3 degrees).

4) Individual reproductive success was measured as the number of nesting times, the number of successful nesting times for each male, the number of hatched fry per nest (represented as RS-index) and RS-index per male.

5) Environmental variability is not a major cause for unsuccessful nesting. Although the causes for unsuccessful nests were not directly examined here, inter-individual interactions greatly controlled the fate of nesting.

6) Body size and environmental factors (water temperature, water depth, changes in water level and possibly food supply) hardly

correlated with reproductive success. The brightness of nuptial coloration at the onset of breeding correlated significantly with individual success. Individual variation in the development of secondary sexual characteristics such as a nuptial coloration may have an important consequence for the lifetime reproductive success of the individuals. There existed no evident relationship between fish density and RS-index.

7) After failing in hatch, males were significantly more likely to move their nest sites than after a successful nest. However, the successive nesting cycle was not always successful.

8) The early or peak breeders tend to have longer breeding cycles than the late breeders. Some plausible reasons for this temporal variation are discussed. One reason was a sex difference in immigration to the breeding sites; males tended to settle and nest before the arrival of females.

9) There was also a lot of variations in nest-site location. The spatial pattern of nest distribution was strongly related to the temporal pattern, because the first males which settled, build their nests more often in those nest-sites along the shore where the nest was covered on one or two sides by vegetation. The location of nest site was significantly correlated with reproductive success.

10) An early initiation of nest-building allows a male to facilitate a high reproductive success. When males nested in partly concealed places along the shore, they could sometimes obtain a high reproductive success irrespective of the date of breeding initiation.

11) There is evidence that early or peak breeders which settled and nested in partly concealed areas along the shore (nest-site type D) could maximize their reproductive success. Although a rate of nesting

success in nest-site type D was not as high as that of nesting success in densely vegetated areas (type E and F), the raiding pressure was lower and the frequency of female visits was higher. Moreover, individual males which nested in groups in type D obtained the highest success.

12) Thus, reproductive success was largely determined not only by the first date of nest-building and nest position, but also by neighbouring nesting linkage. Long-lasting stable relationships between synchronous individuals allow the development of many complex aspects, such as a neighbouring nesting linkage, of the social system. Male which nested in a link experienced both fewer intrusions and more female visits. Nests in link resulted from an increased rate of nesting success as compared to that in non-link.

13) In conclusion, the reproductive success of a male depended on the number of nests he built, the number of females he mated and the number of eggs he fertilized. These were all strongly influenced by the date of nest-building initiation, nest-site characteristics, and link nesting.

14) The present study was described that raiding behaviour included stolen fertilizations, egg-stealing and egg-cannibalism. Each raiding behaviour was discussed and its possible ecological significance indicated. I also discussed the issue of surplus males when the individual life time is taken into account.

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Fig. 1

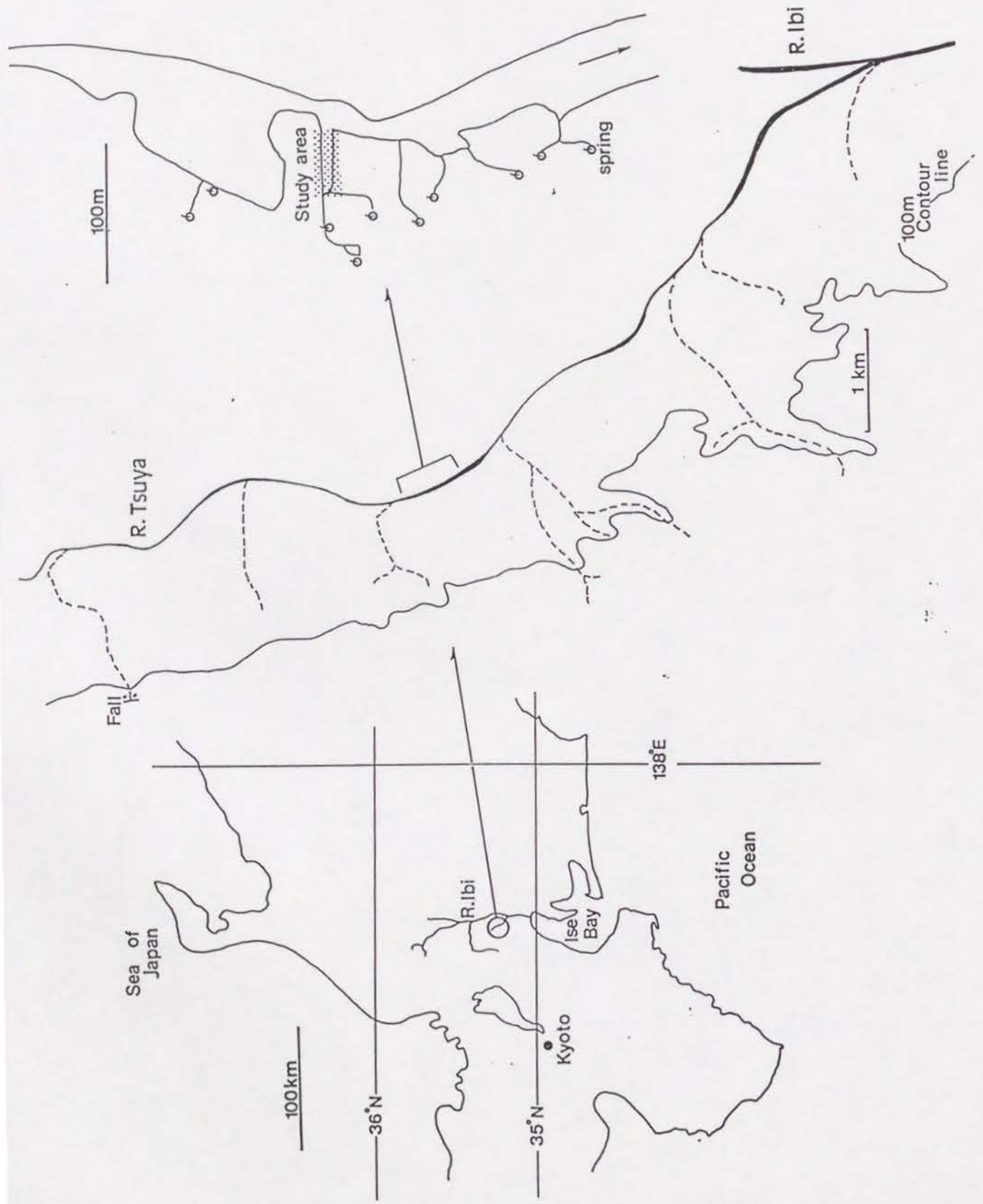

R. Ibi
Study area
spring
100m
100m Contour line
1 km
R. Tsuya
Fall
Sea of Japan
R. Ibi
Ise Bay
Pacific Ocean
138°E
36°N
35°N
Kyoto
100km

Fig. 2

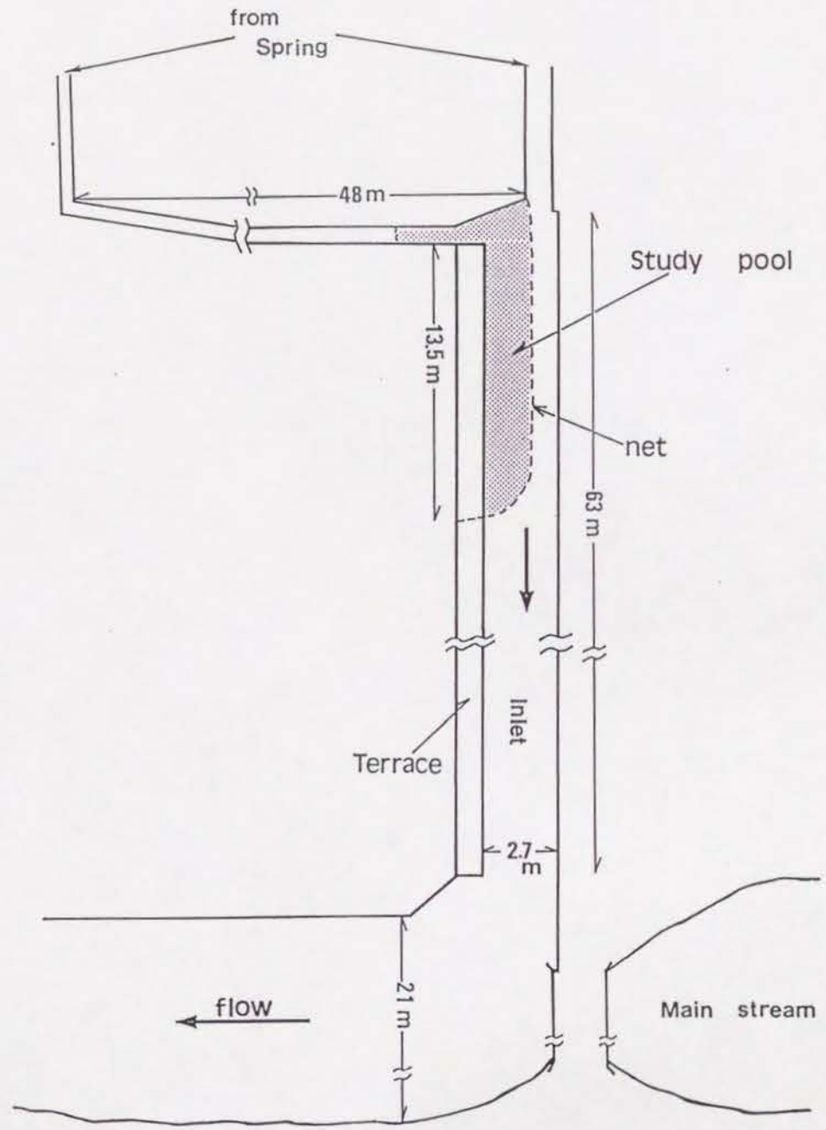

from Spring
48 m
Study pool
13.5 m
net
63 m
Inlet
Terrace
2.7 m
flow
21 m
Main stream

Fig. 3

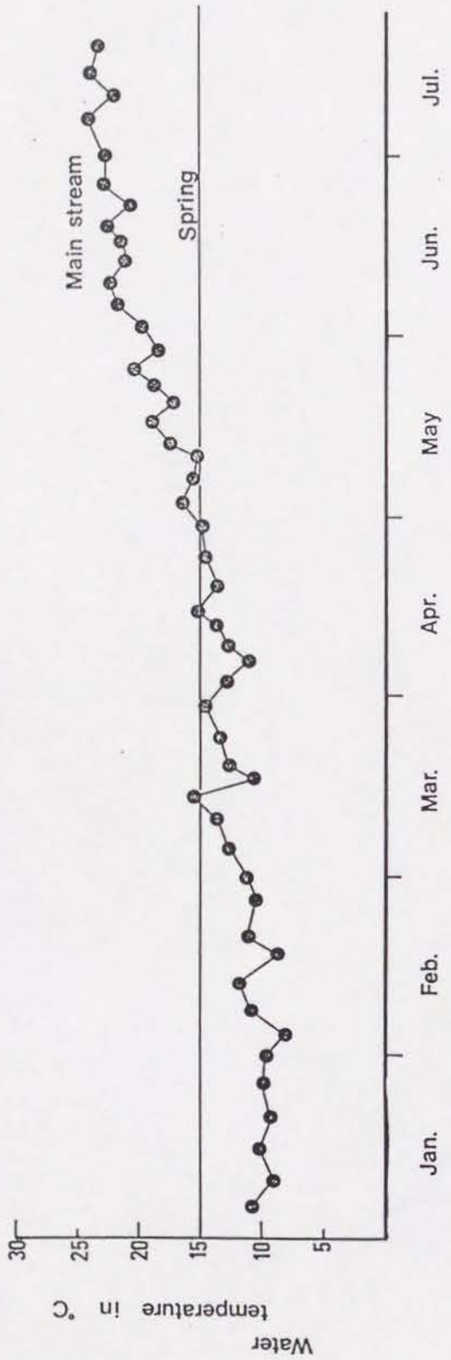

Water temperature in °C
30
25
20
15
10
5
Main stream
Spring
Jan.
Feb.
Mar.
Apr.
May
Jun.
Jul.

Fig. 4

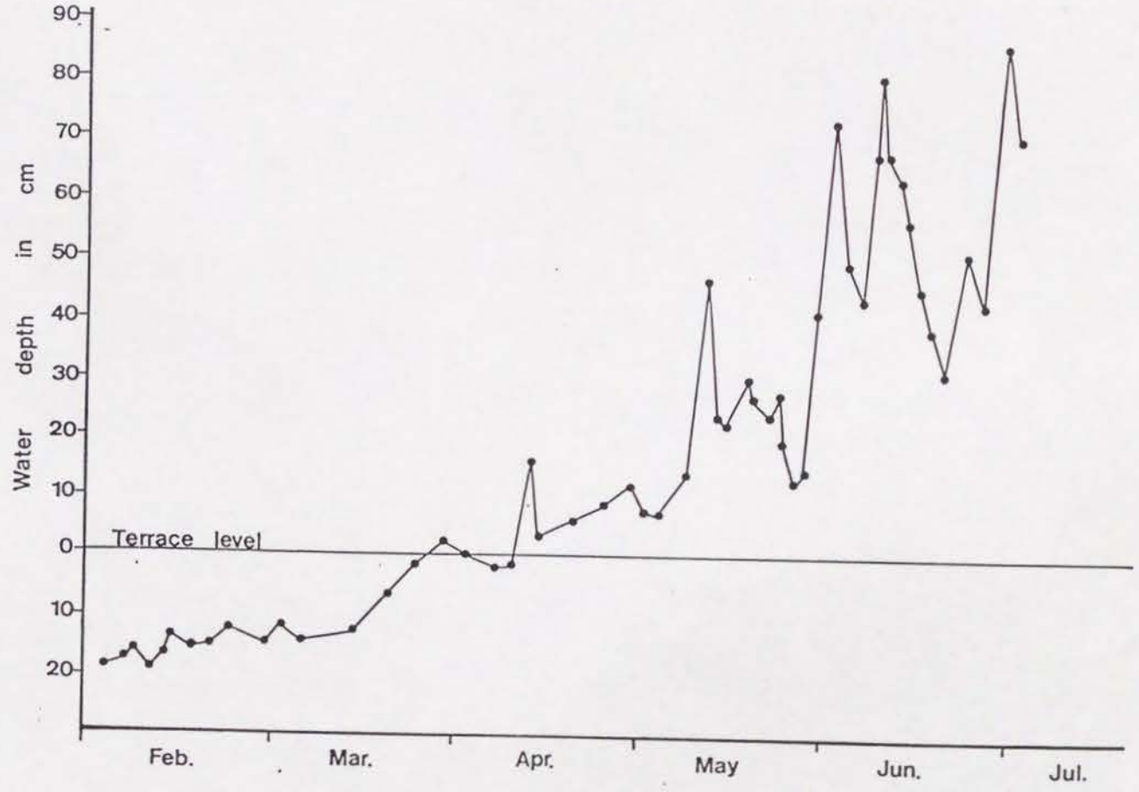

Water depth in cm
90
80
70
60
50
40
30
20
10
0
10
20
Terrace level
Feb.
Mar.
Apr.
May
Jun.
Jul.

Fig. 5

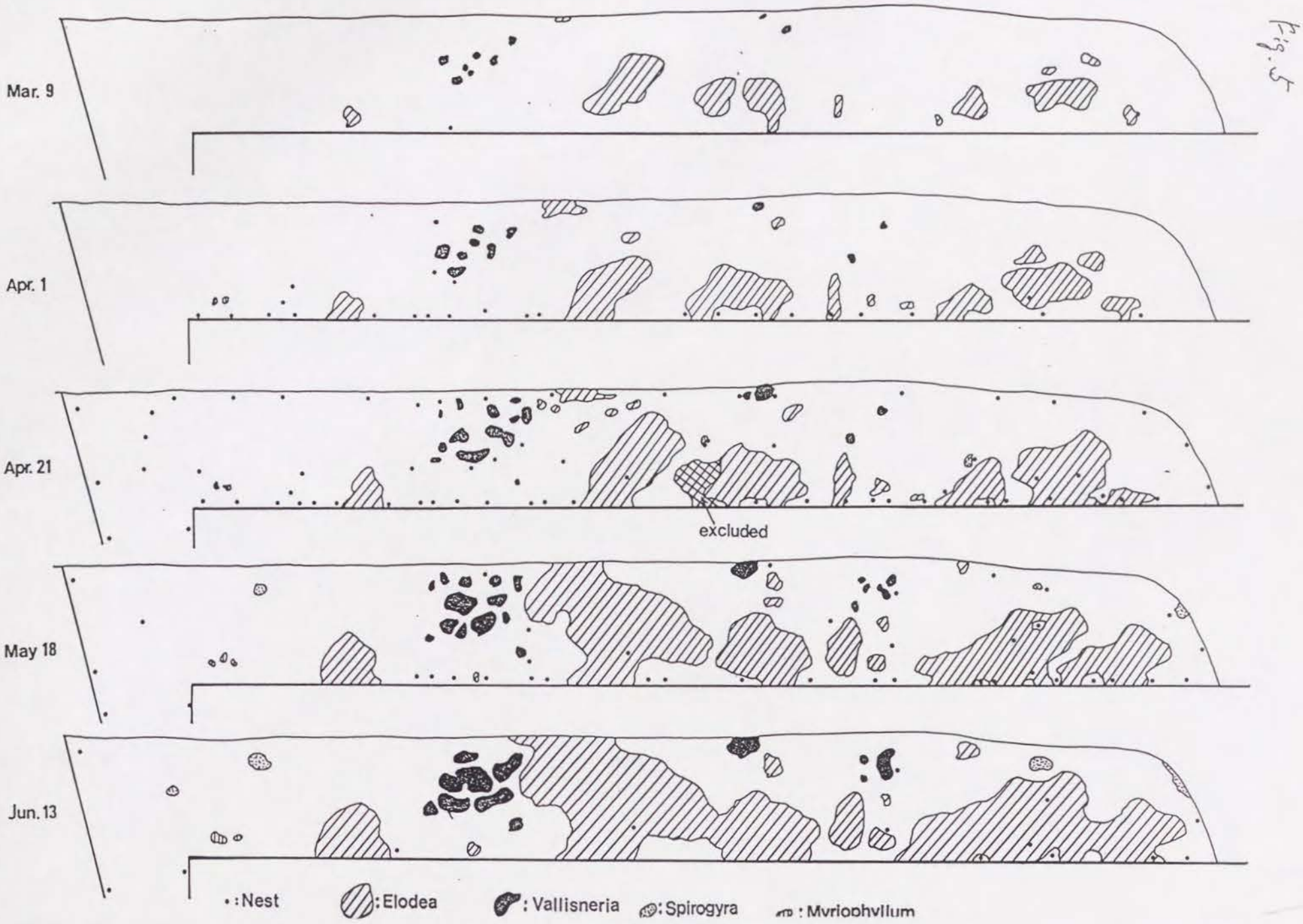

Mar. 9
Apr. 1
Apr. 21
excluded
May 18
Jun. 13
•: Nest
: Elodea
: Vallisneria
: Spirogyra
: Myriophyllum

Fig. 6

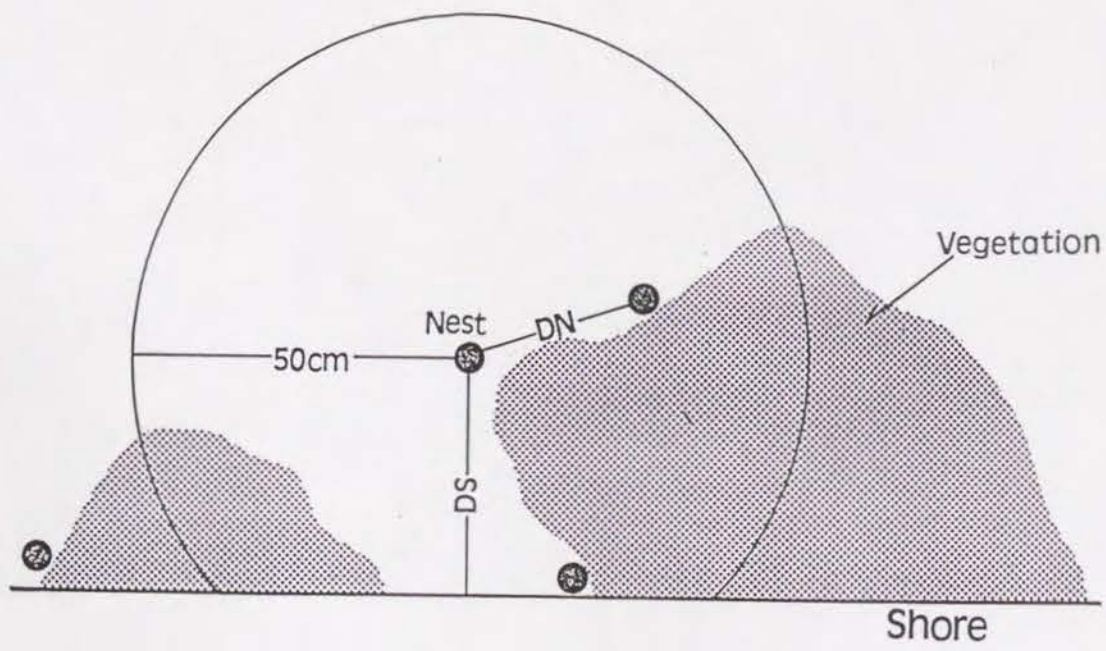

Vegetation
Nest
DN
50cm
DS
Shore

Fig. 7

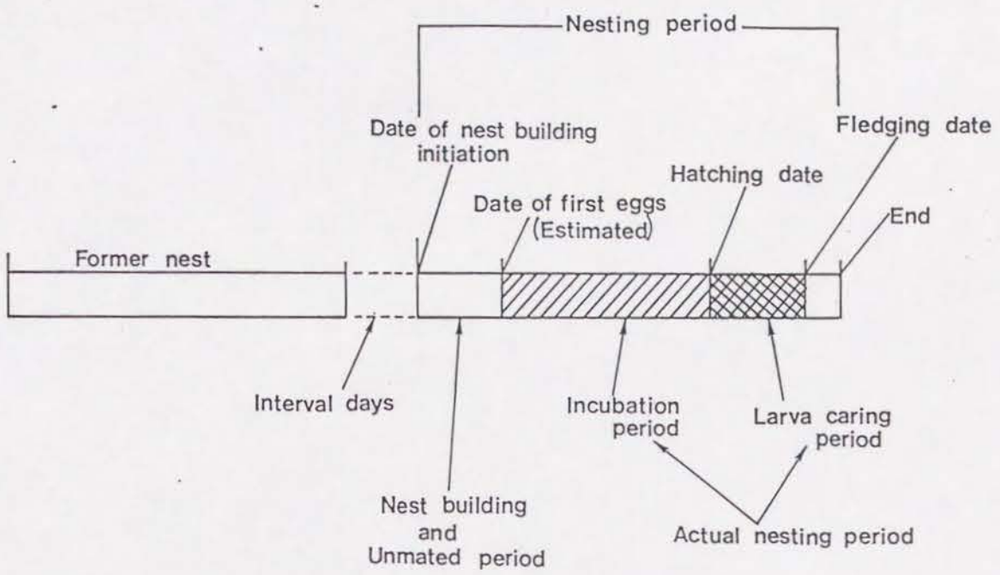

Nesting period
Date of nest building initiation
Fledging date
Hatching date
Date of first eggs (Estimated)
End
Former nest
Interval days
Incubation period
Larva caring period
Nest building and Unmated period
Actual nesting period

Fig. 8

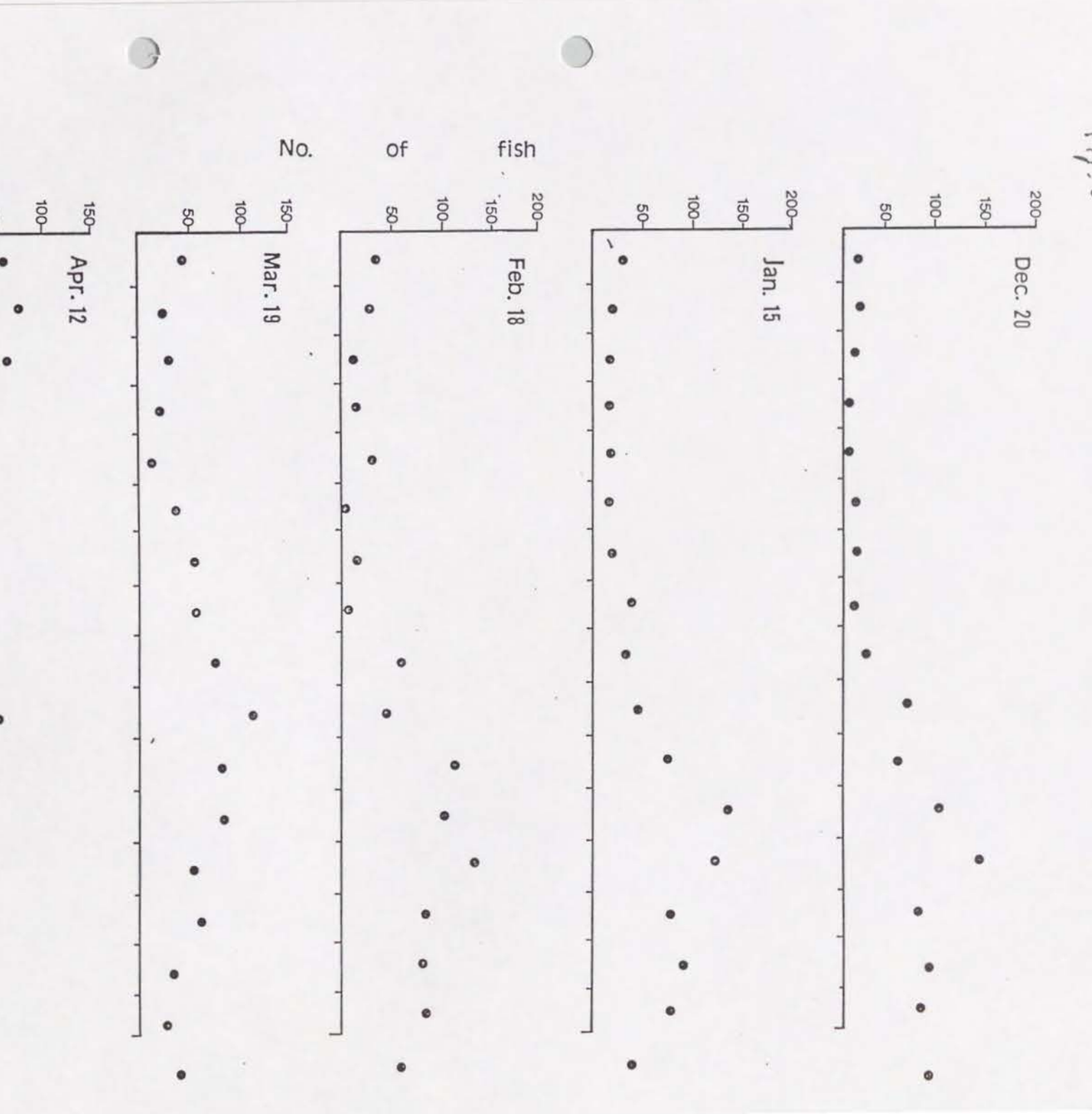

Dec. 20
Jan. 15
Feb. 18
Mar. 19
Apr. 12
No. of fish
200
150
100
50
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 48 R
Section between piles

Fig. 9

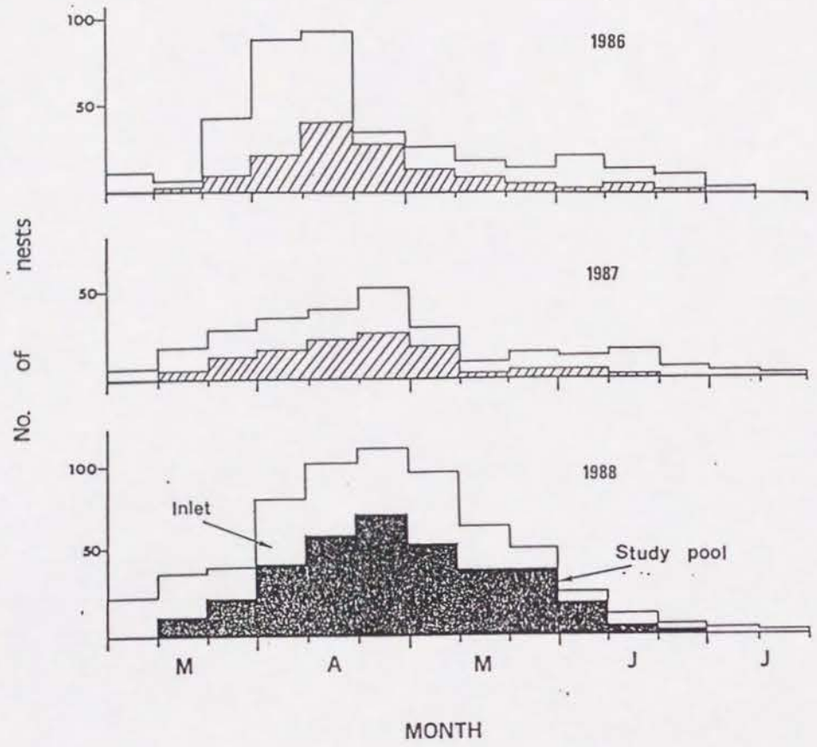

No. of nests
100
50
1986
1987
1988
Inlet
Study pool
M
A
M
J
J
MONTH

Fig. 10

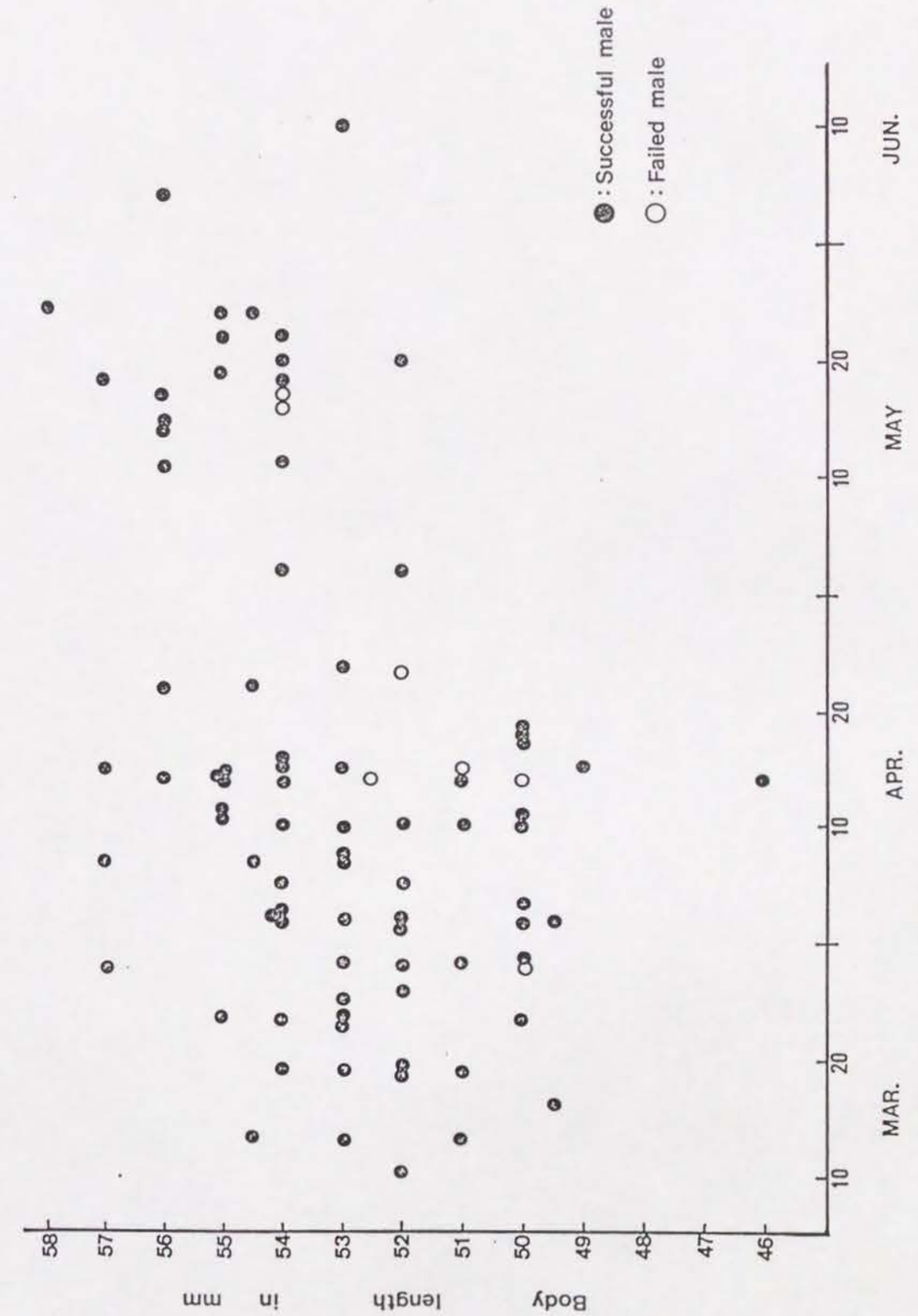

●: Successful male
○: Failed male
Body length in mm
58
57
56
55
54
53
52
51
50
49
48
47
46
10
20
10
20
10
20
MAR.
APR.
MAY
JUN.
D. N. I.

Fig. 11

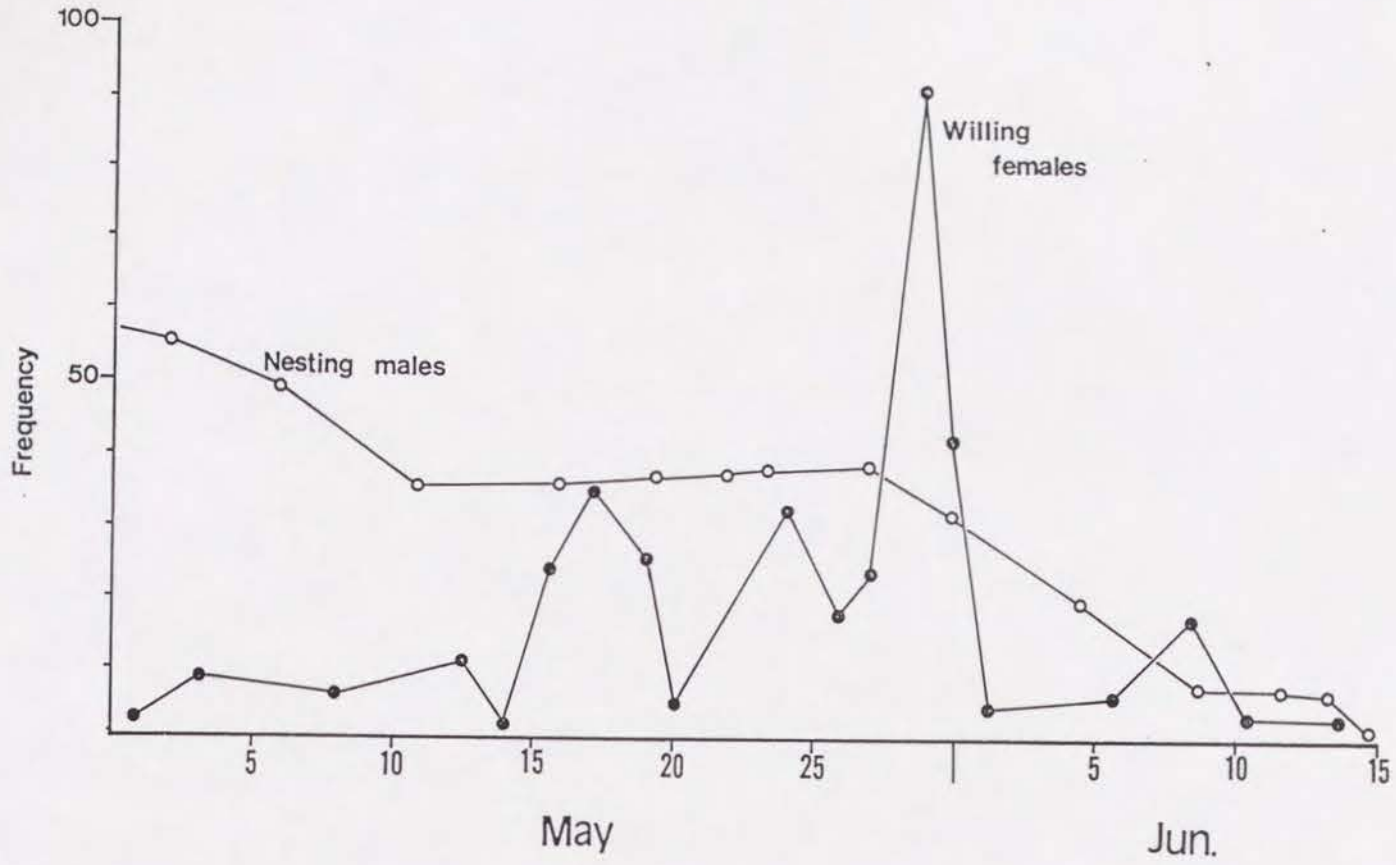

100
50
Frequency
Nesting males
Willing
females
5
10
15
20
25
5
10
15
May
Jun.

Fig. 12

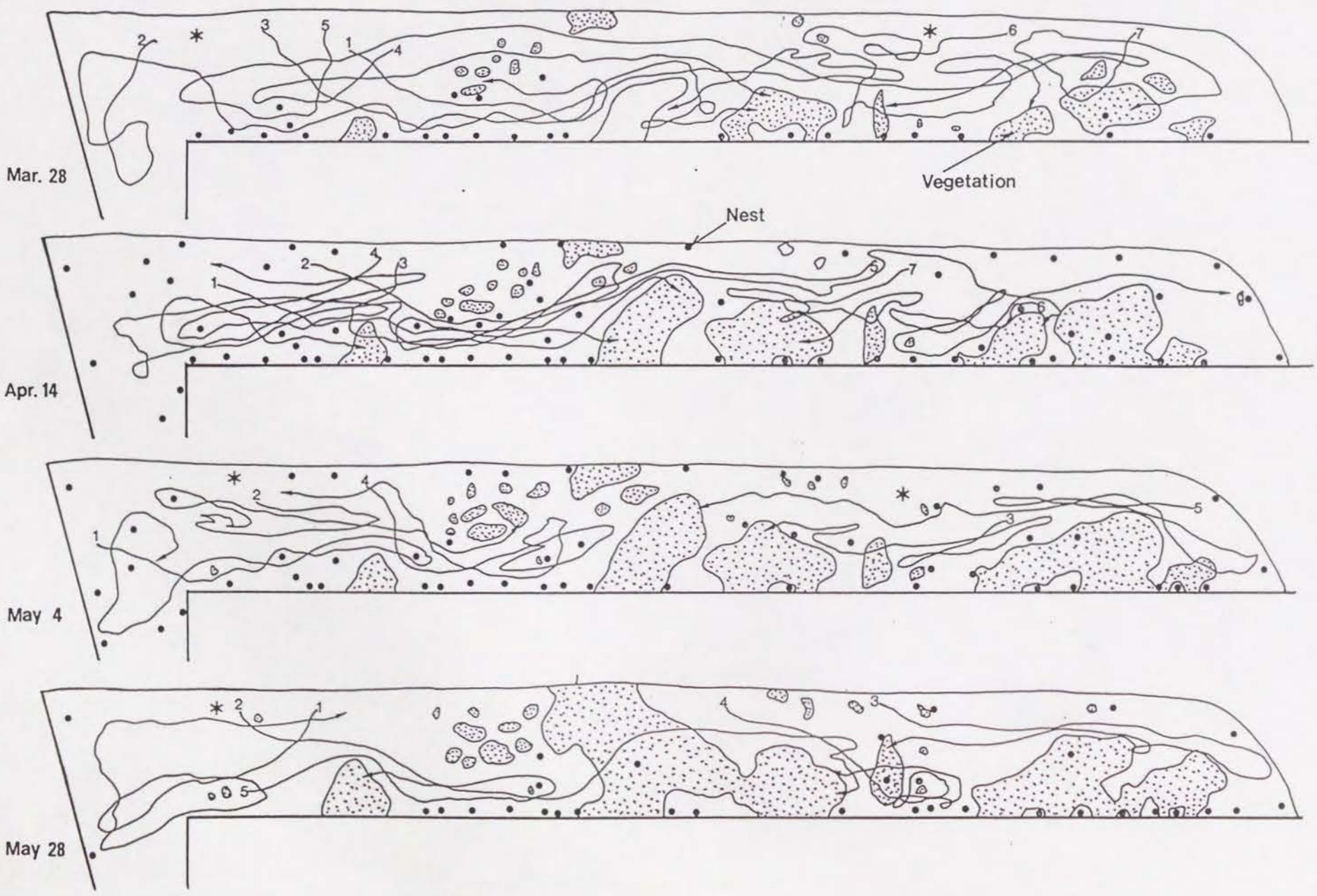

Mar. 28
Vegetation
Nest
Apr. 14
May 4
May 28

Fig. 13

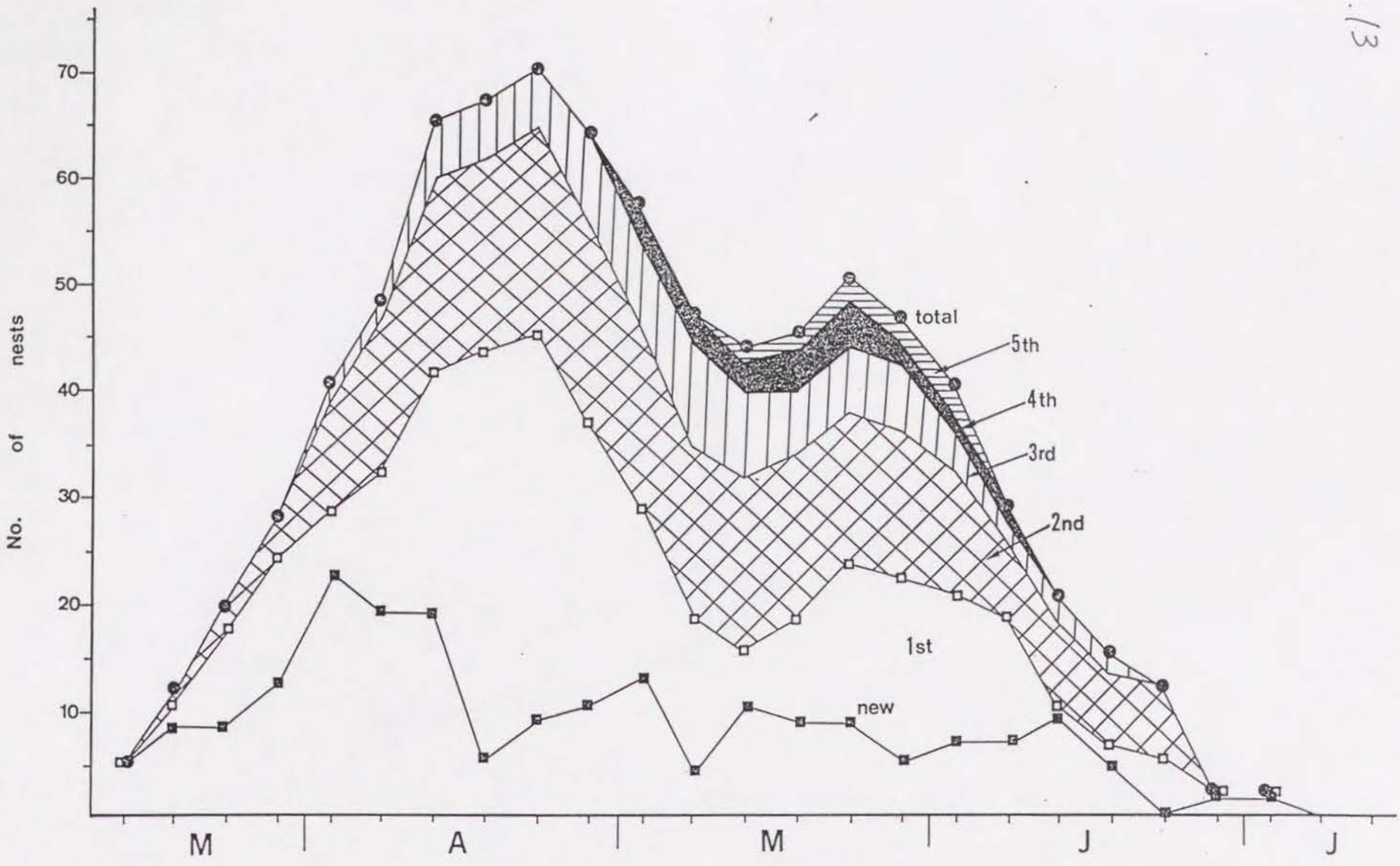

No. of nests
70
60
50
40
30
20
10
total
5th
4th
3rd
2nd
1st
new
M
A
M
J
J

Fig. 14

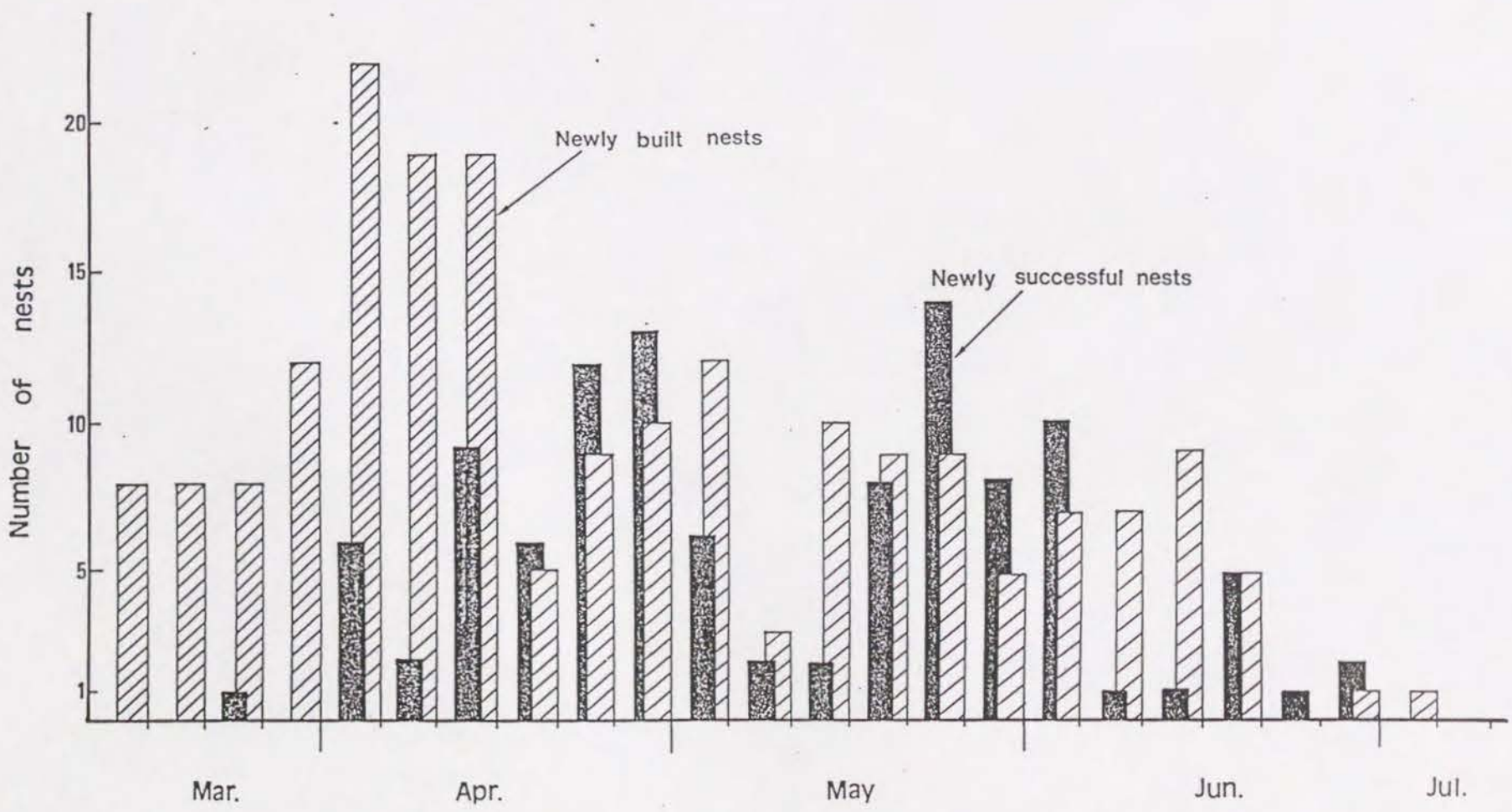

Number of nests
20
15
10
5
1
Newly built nests
Newly successful nests
Mar.
Apr.
May
Jun.
Jul.

Fig. 15

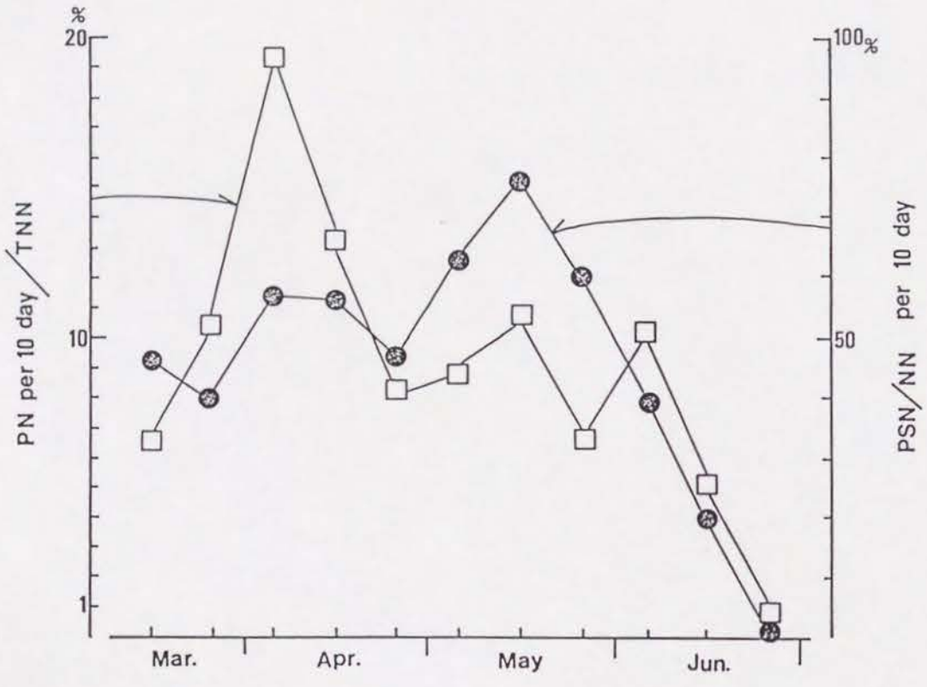

%
20
10
1
100%
50
PN per 10 day / TNN
PSN/NN per 10 day
Mar.
Apr.
May
Jun.

Fig. 16

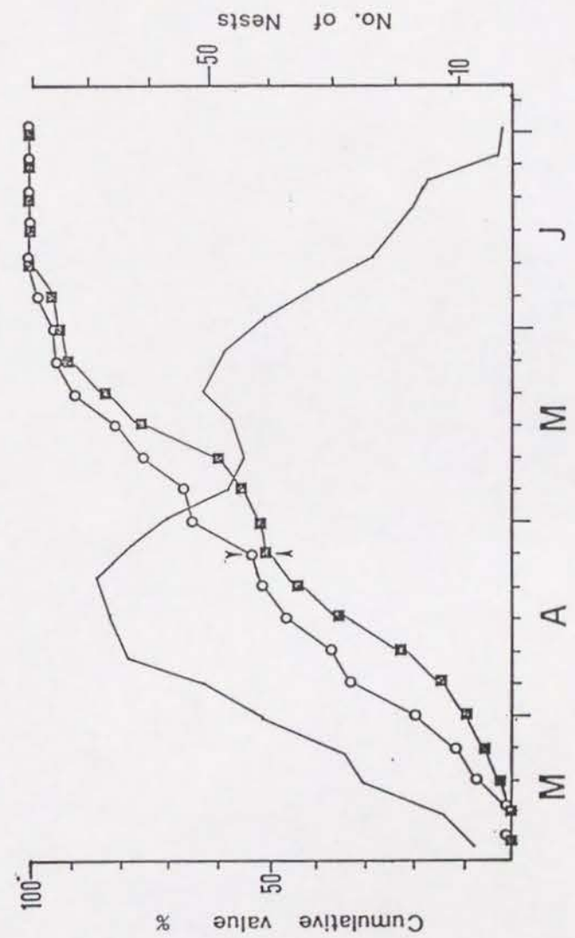
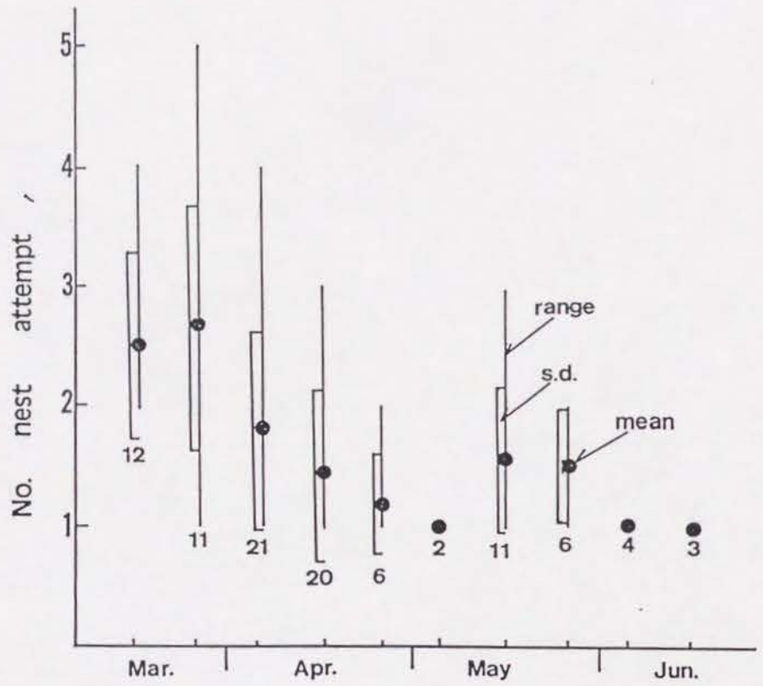
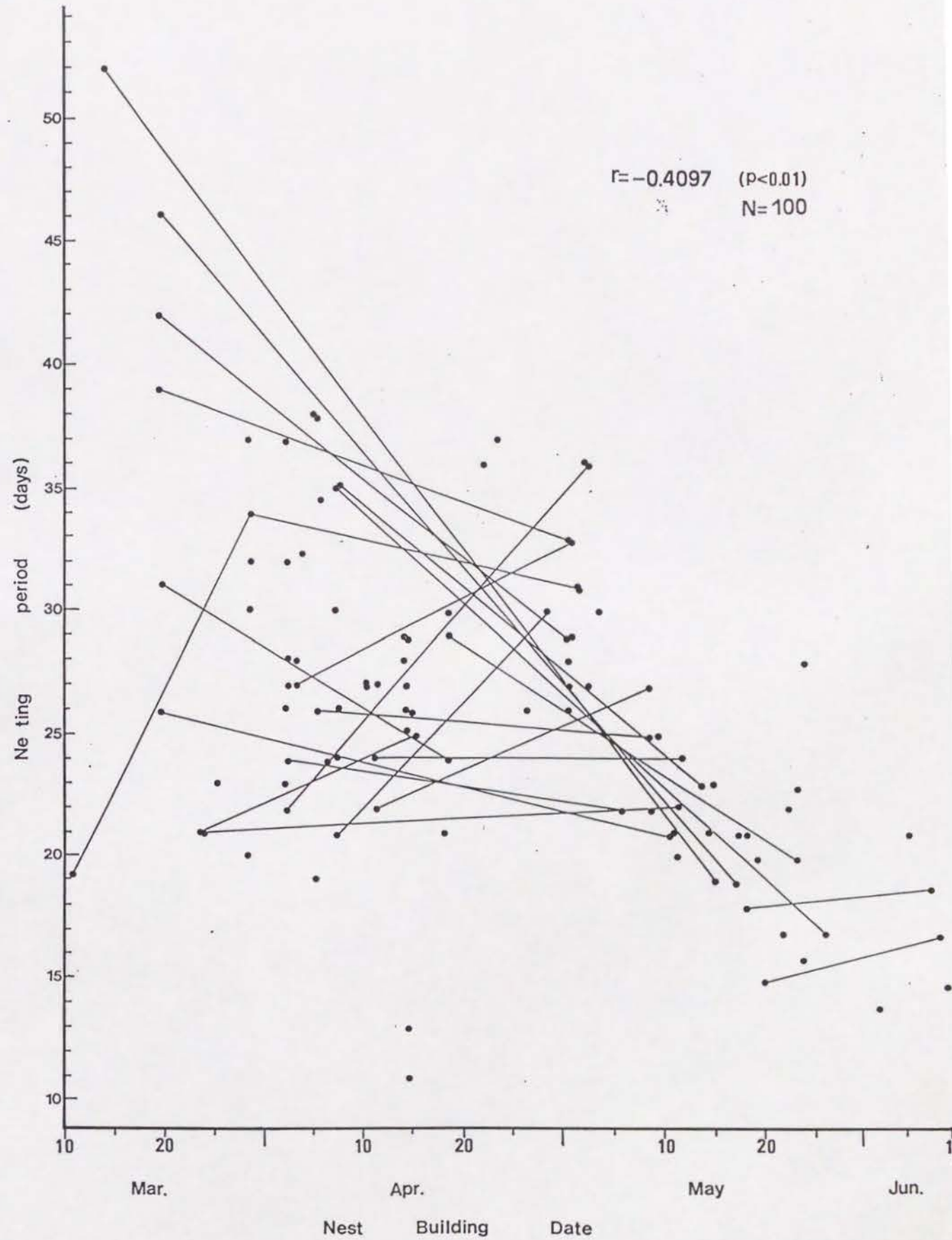

No. of Nests
50
10
J
M
A
M
Month
Cumulative value %
100
50

Fig. 17



D. N. I.

Fig. 18



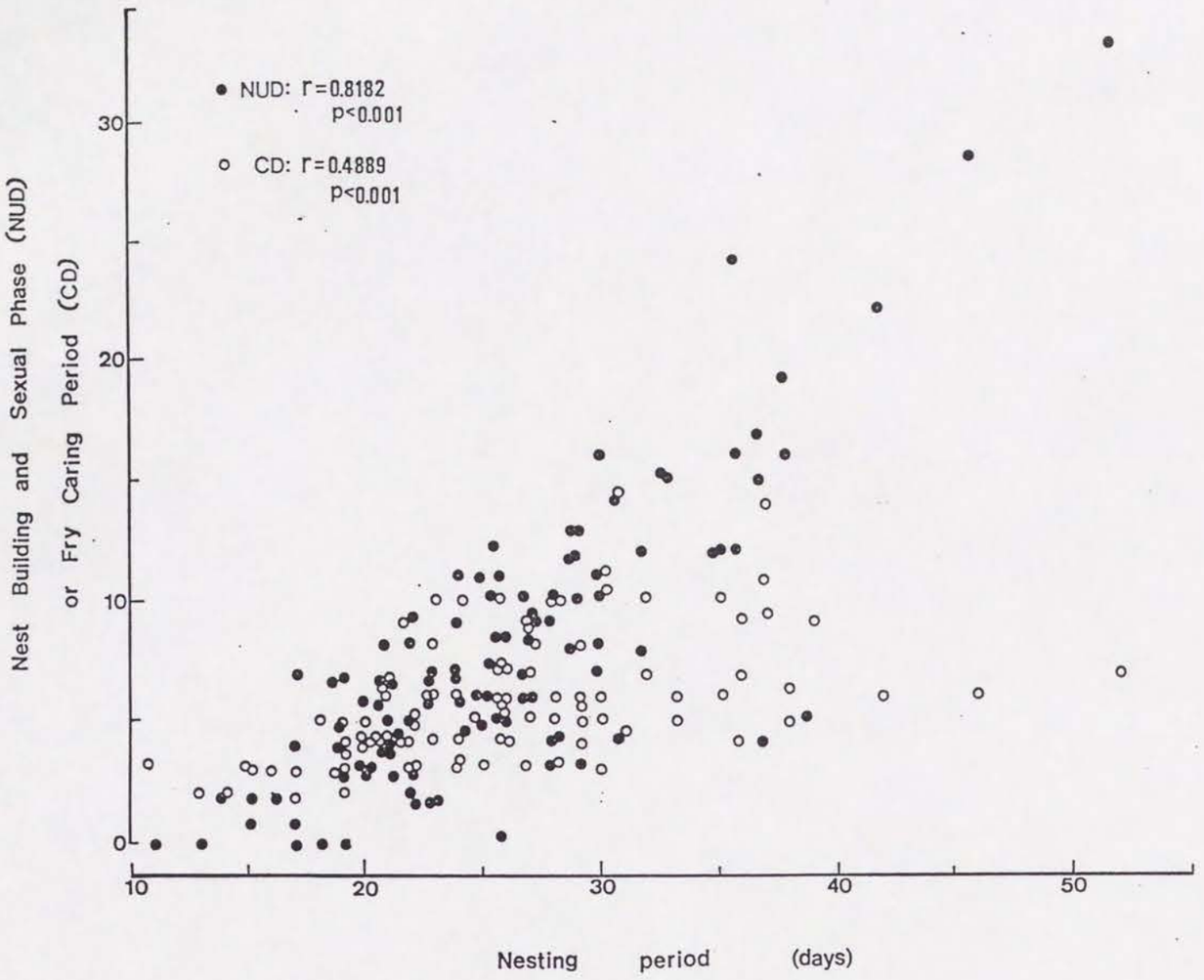

● NUD: r=0.8182
p<0.001
○ CD: r=0.4889
p<0.001
Nest Building and Sexual Phase (NUD)
or Fry Caring Period (CD)
0
10
20
30
10
20
30
40
50
Nesting period (days)

Fig. 20

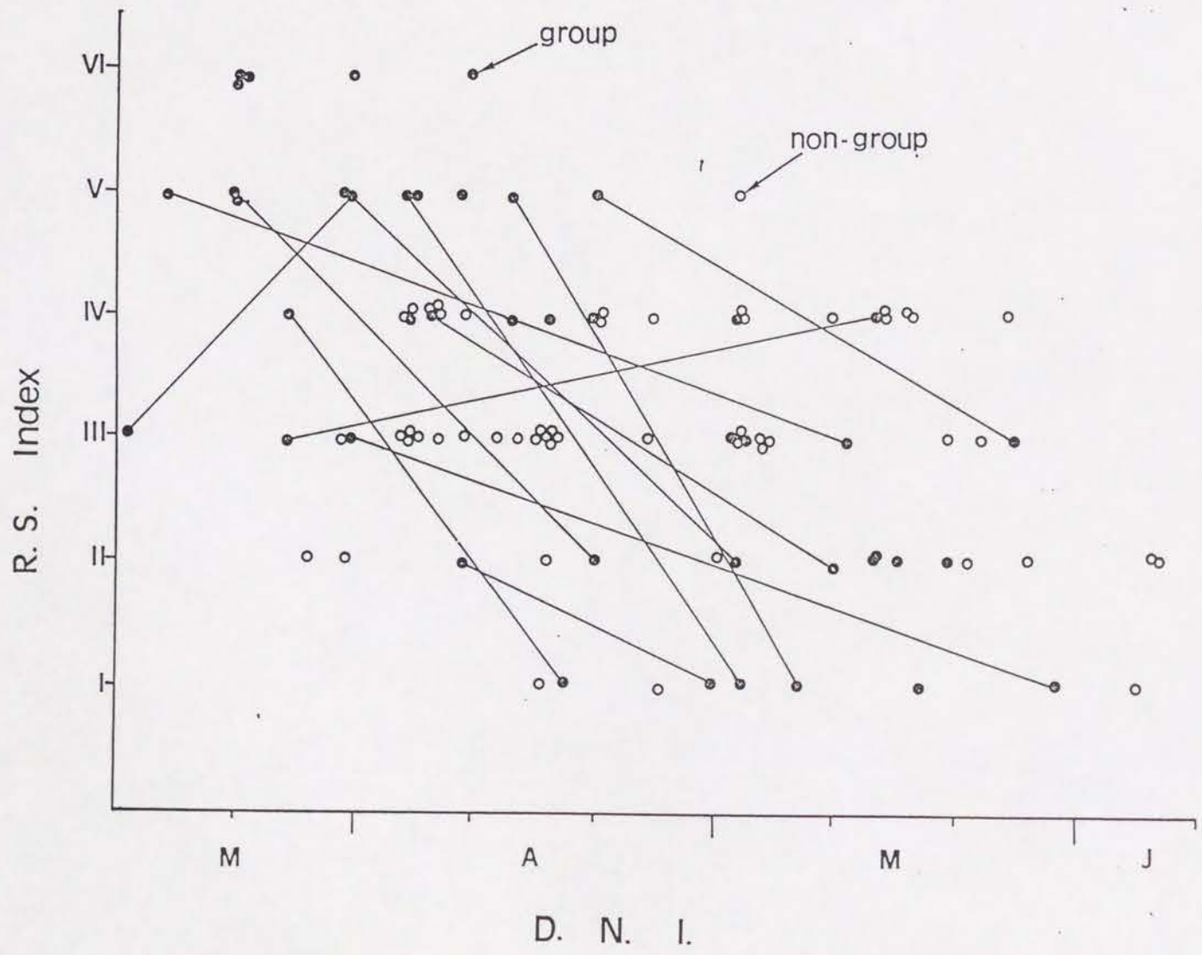

group
non-group
R. S. Index
VI
V
IV
III
II
I
M
A
M
J
D. N. I.

Fig. 21

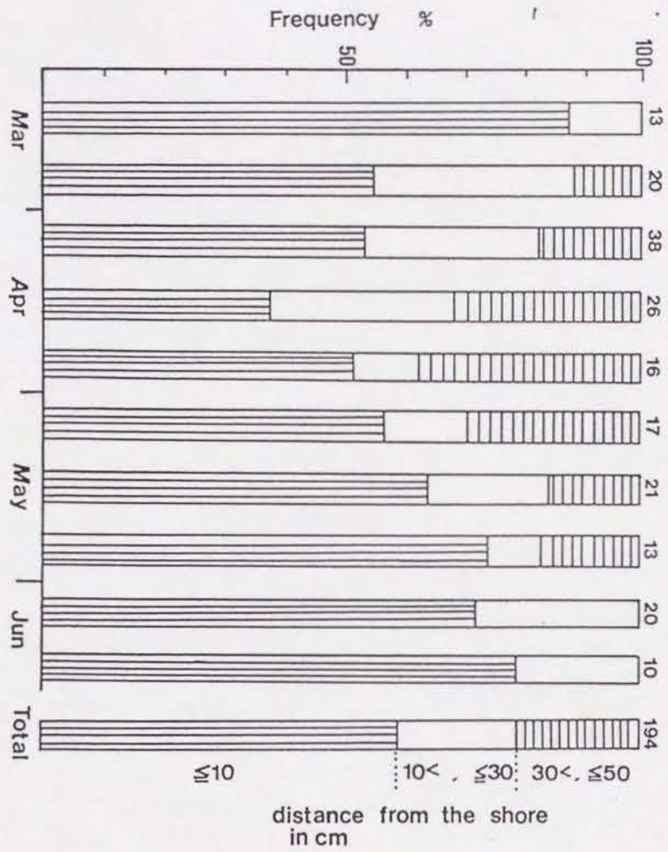

Frequency %
50
100
Mar
Apr
May
Jun
Total
13
20
38
26
16
17
21
13
20
10
194
≦10
10< , ≦30
30< , ≦50
distance from the shore
in cm

Fig. 22

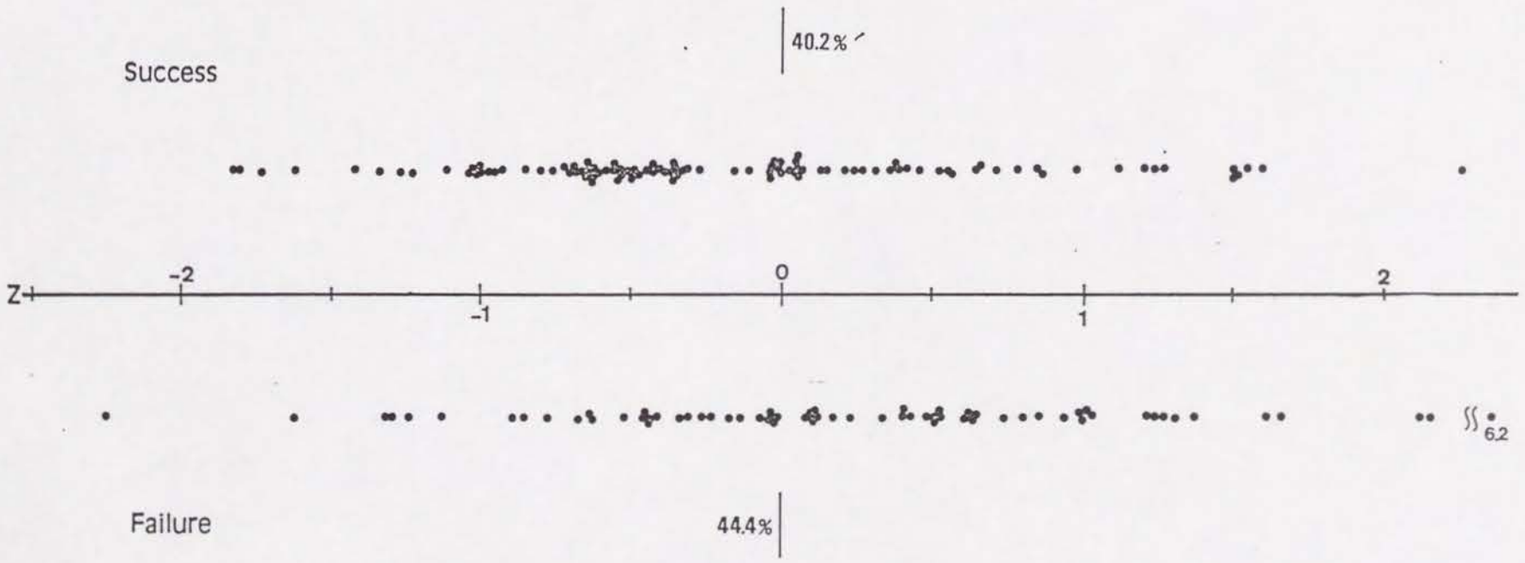

40.2%
Success
Z
-2
-1
0
1
2
6.2
Failure
44.4%

Fig. 23

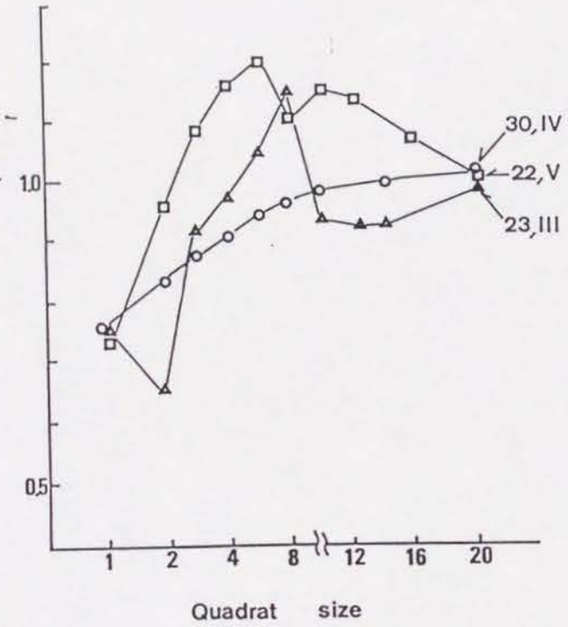
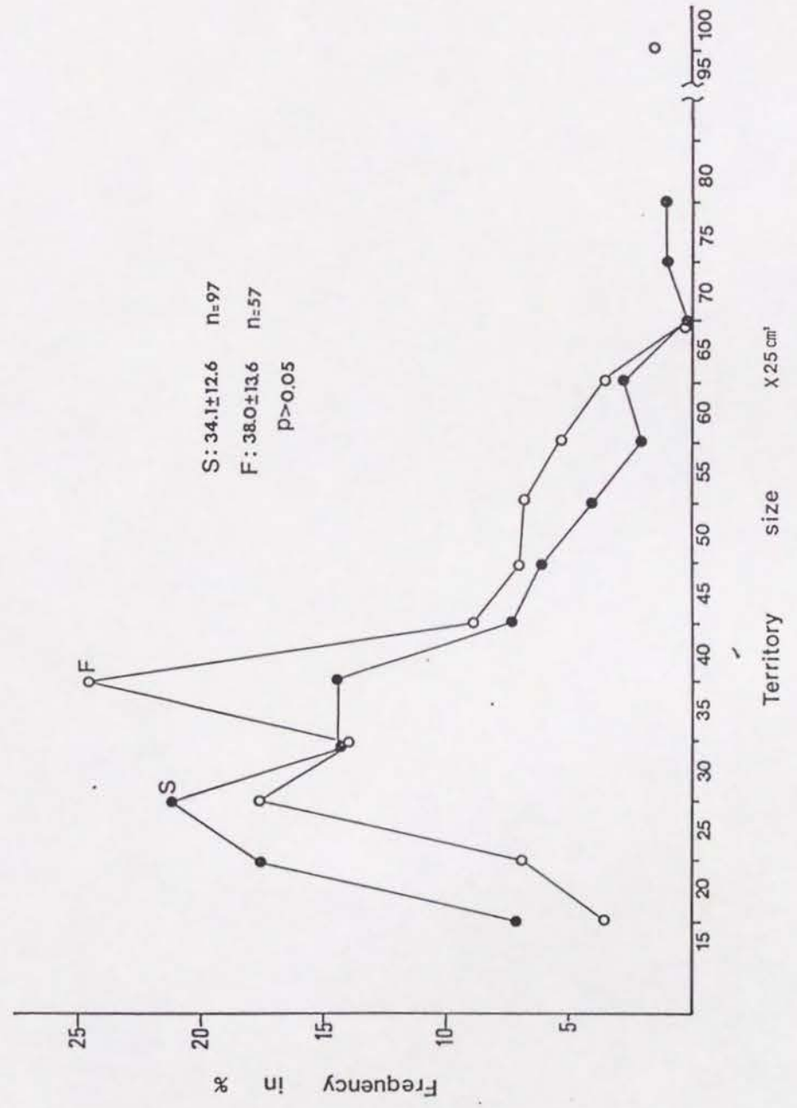
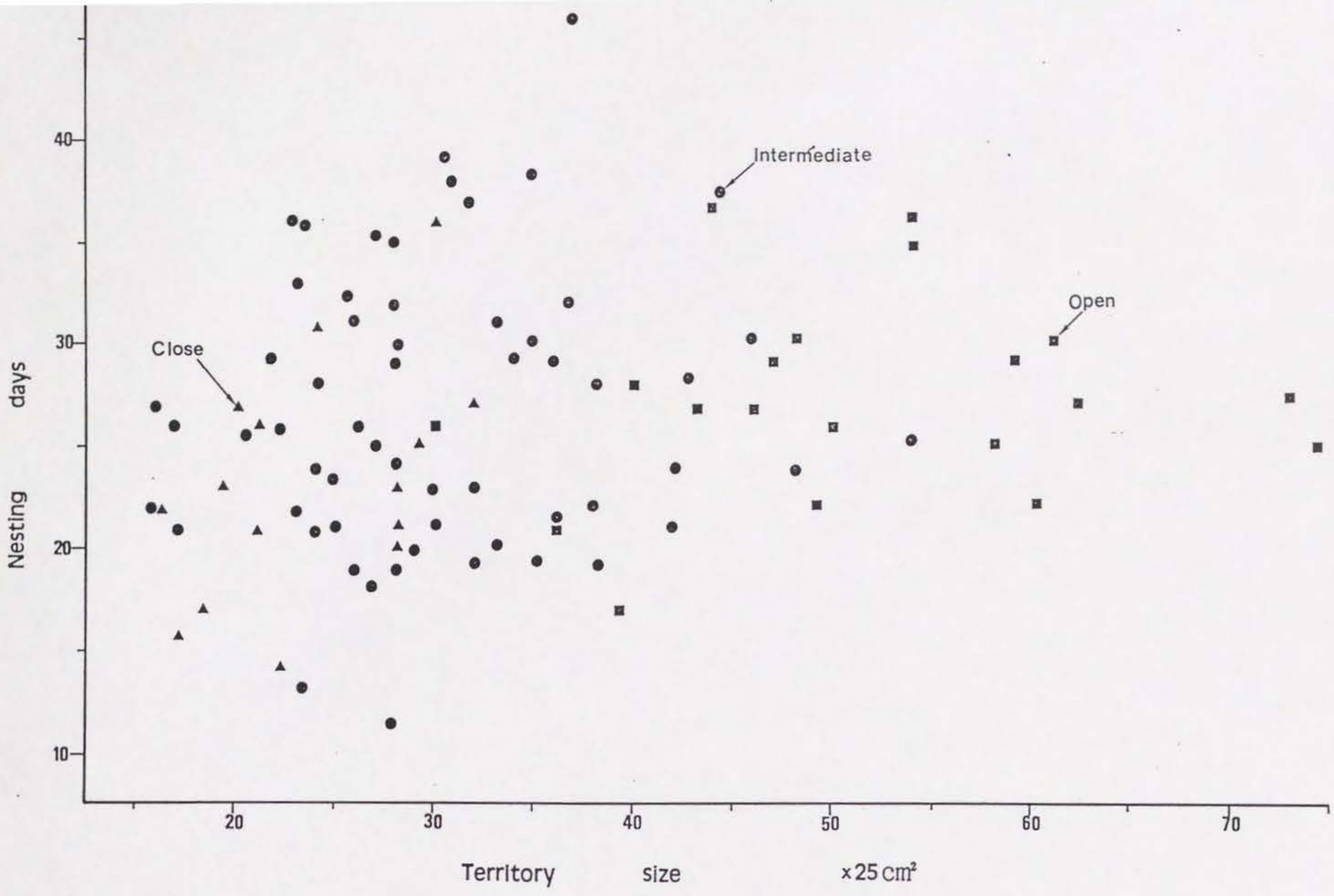
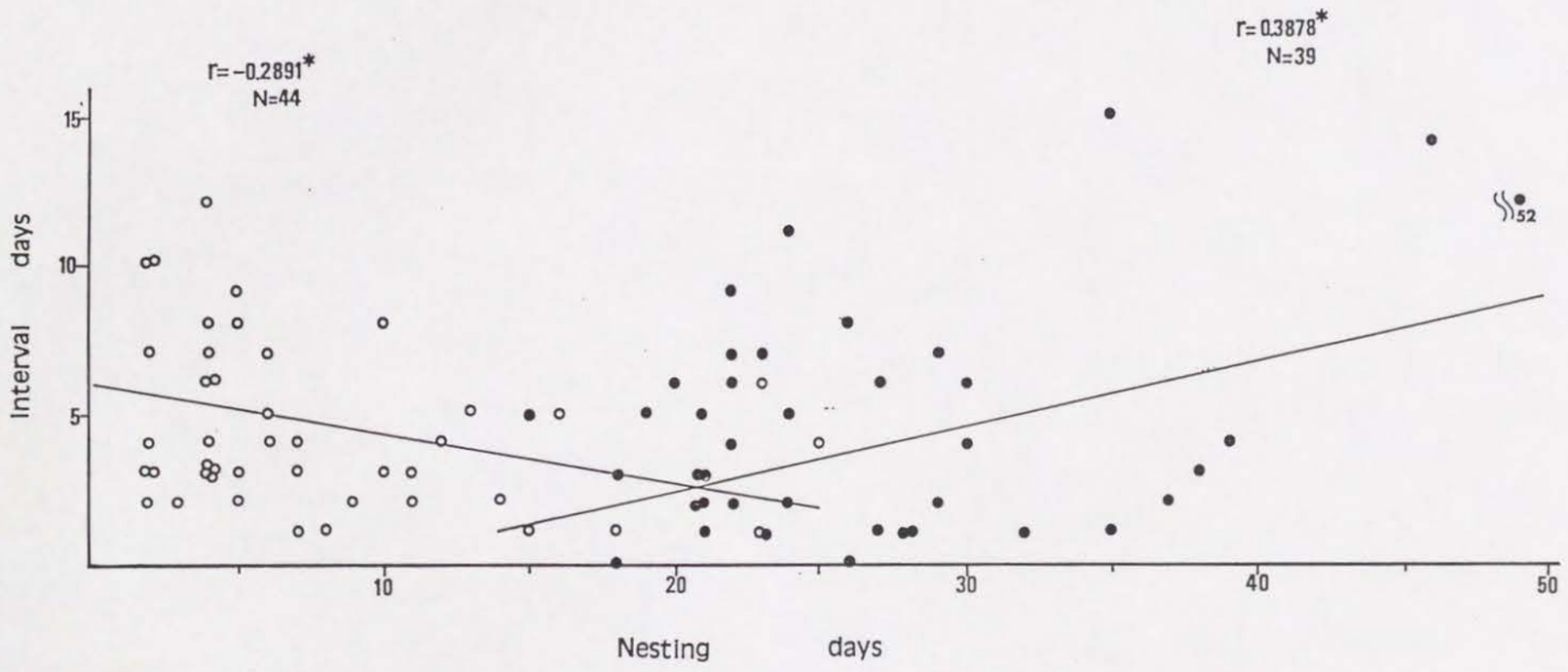
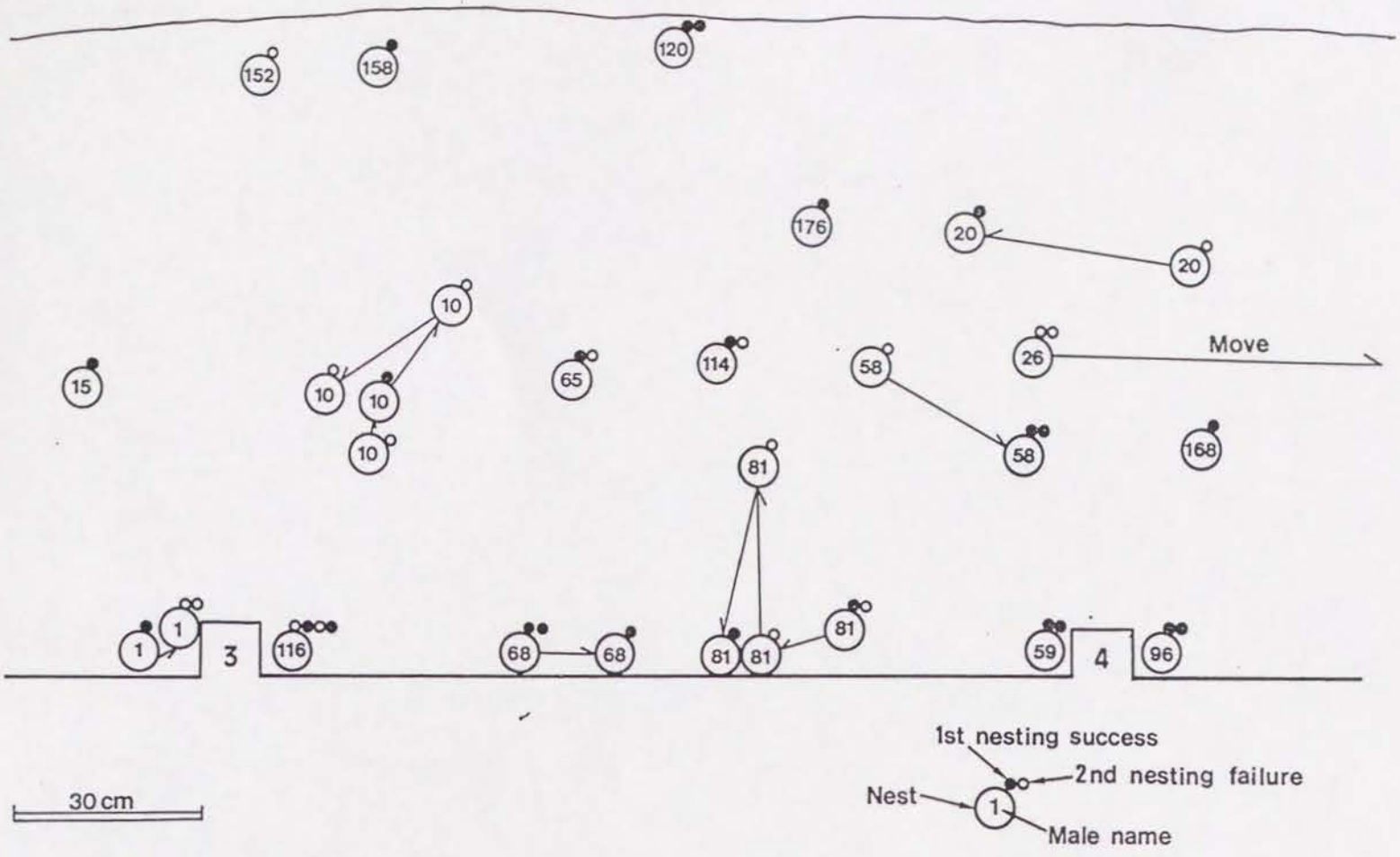

Iδ
1.0
0.5
30, IV
22, V
23, III
1
2
4
8
12
16
20
Quadrat size

Fig. 24


S: 34.1±12.6 n=97
F: 38.0±13.6 n=57
p>0.05
F
S
25
20
15
10
5
Frequency in %
15
20
25
30
35
40
45
50
55
60
65
70
75
80
95
100
Territory size X 25 cm²


Intermediate
Open
Close
Nesting days
10
20
30
40
20
30
40
50
60
70
Territory size x25 cm²


r= −0.2891*
N=44
r= 0.3878*
N=39
52
Interval days
5
10
15
10
20
30
40
50
Nesting days


152
158
120
176
20
20
10
10
10
10
15
65
114
58
26
Move
58
168
81
1
1
3
116
68
68
81
81
81
59
4
96
30 cm
1st nesting success
2nd nesting failure
Nest
1
Male name

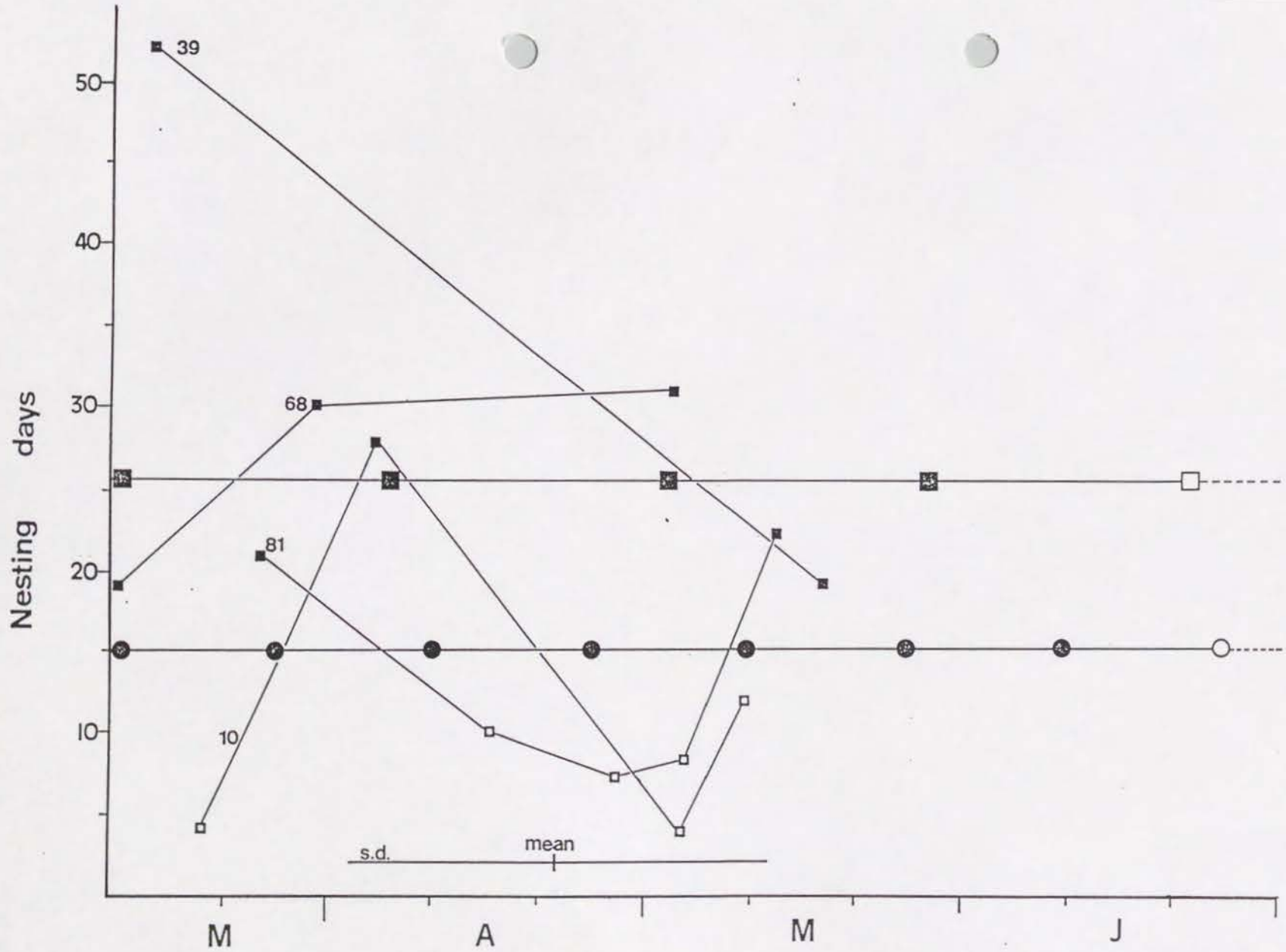
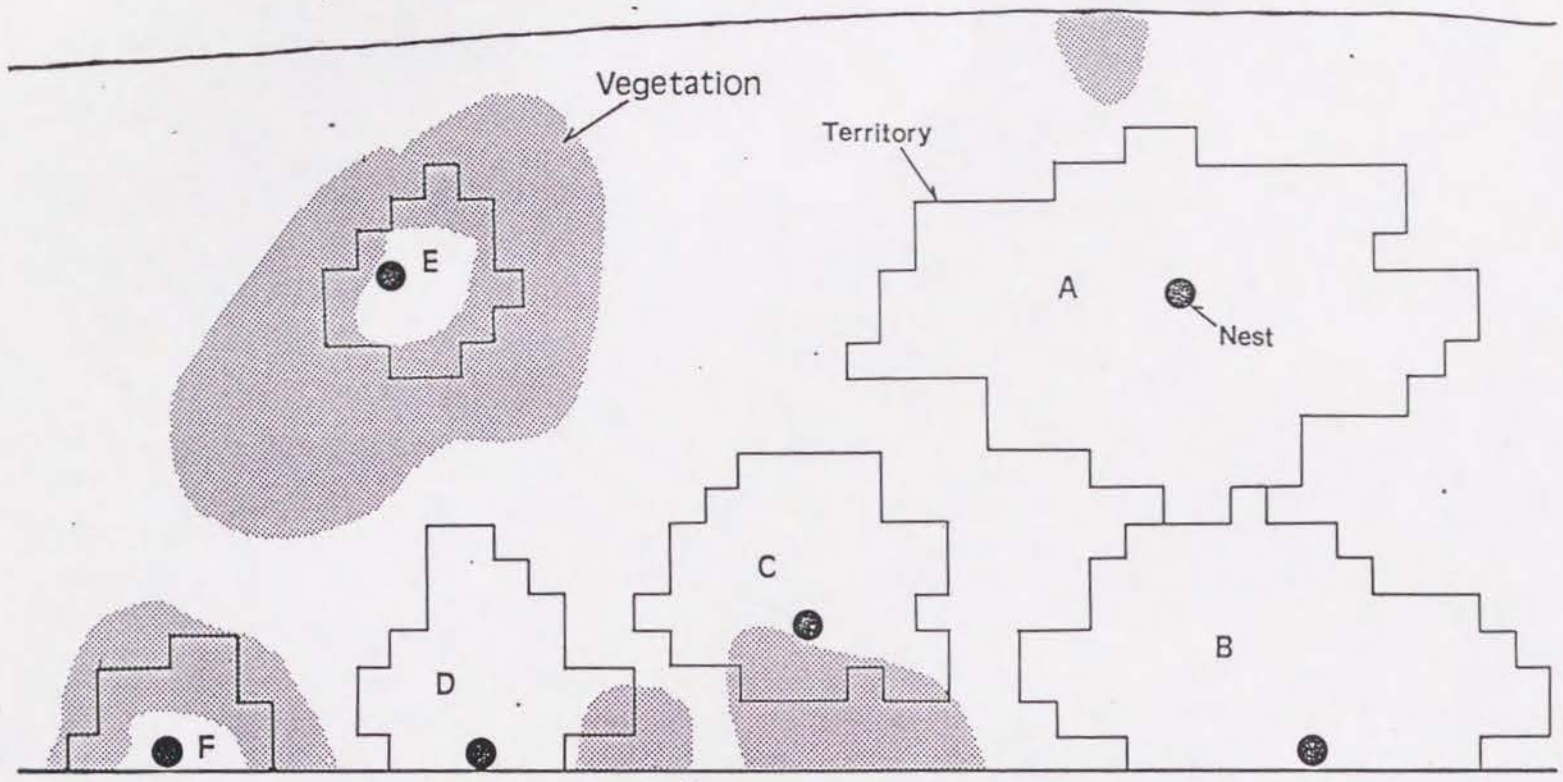

39
50
40
30
20
10
68
81
10
s.d.
mean
Nesting days
M
A
M
J
Initiate date

Fig. 28


Vegetation
Territory
E
A
Nest
C
B
D
F

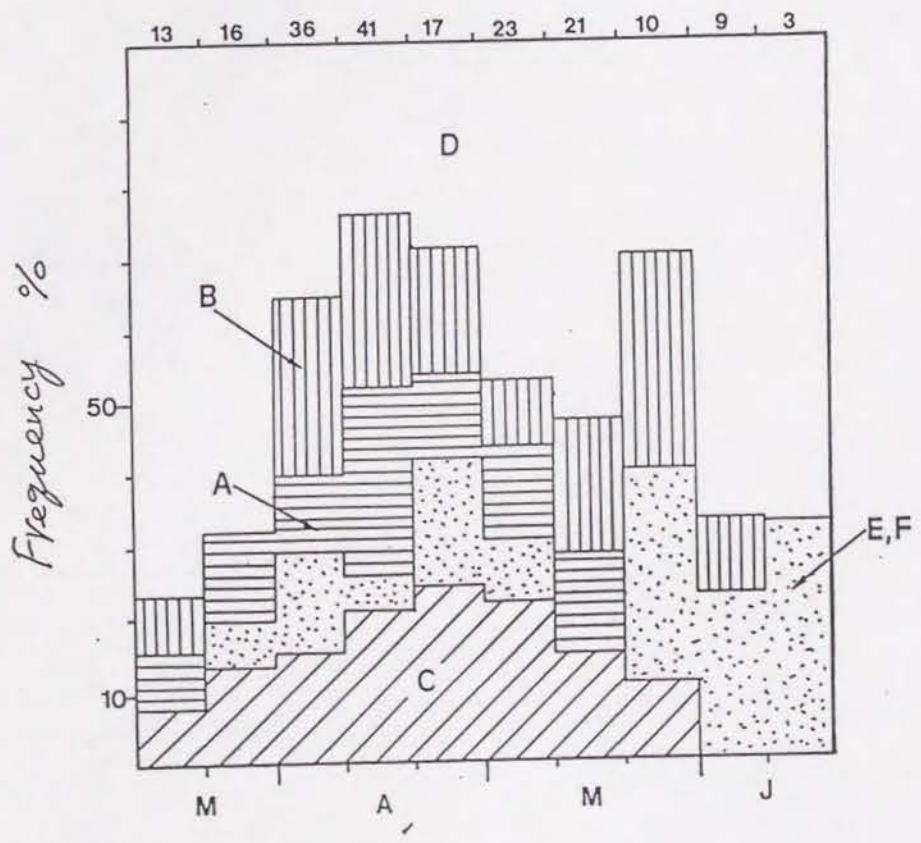

13 16 36 41 17 23 21 10 9 3
D
B
A
E,F
C
50
10
Frequency %
M
A
M
J

Fig. 30.

Fig.31

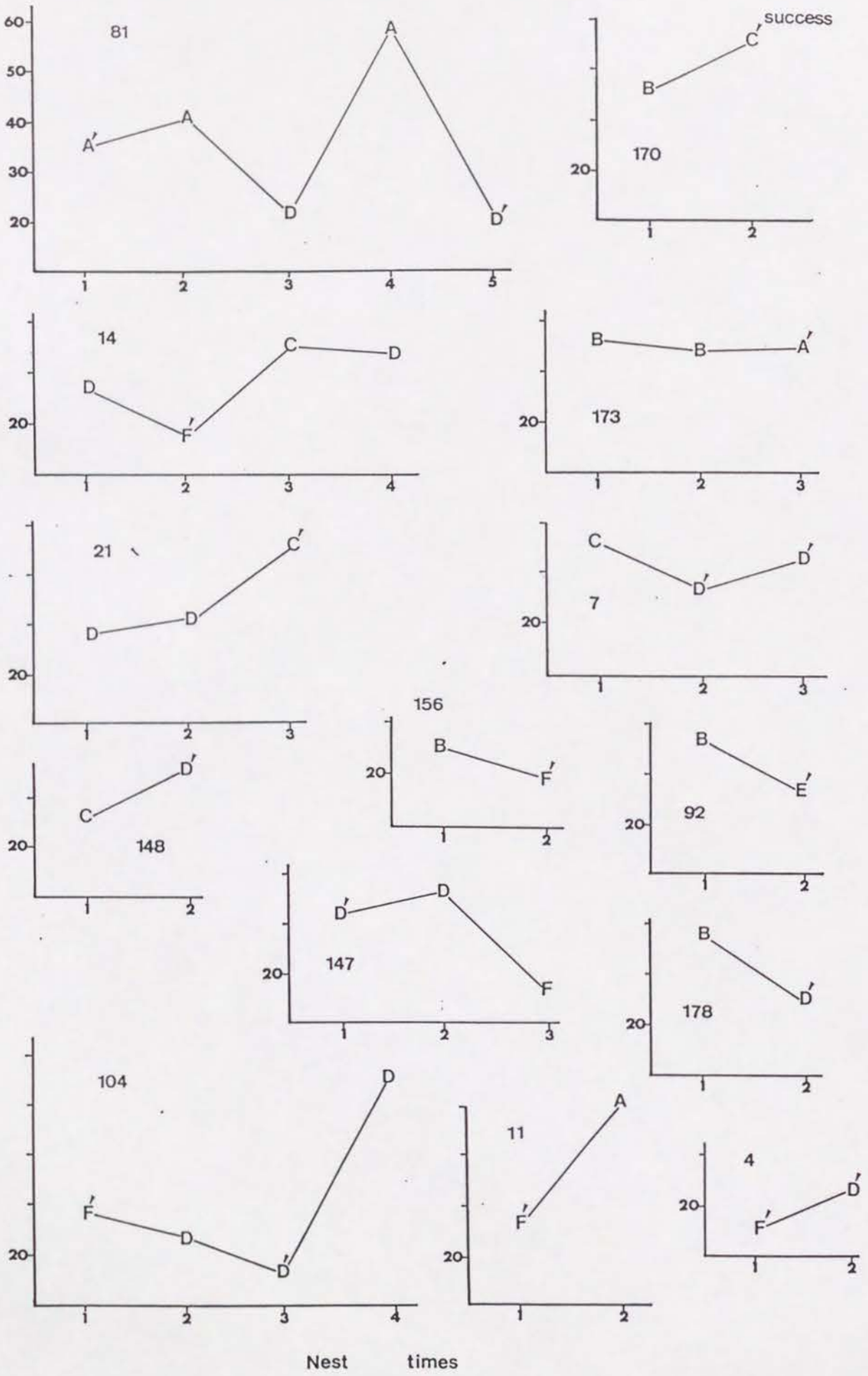


Fig.32

Fig.32

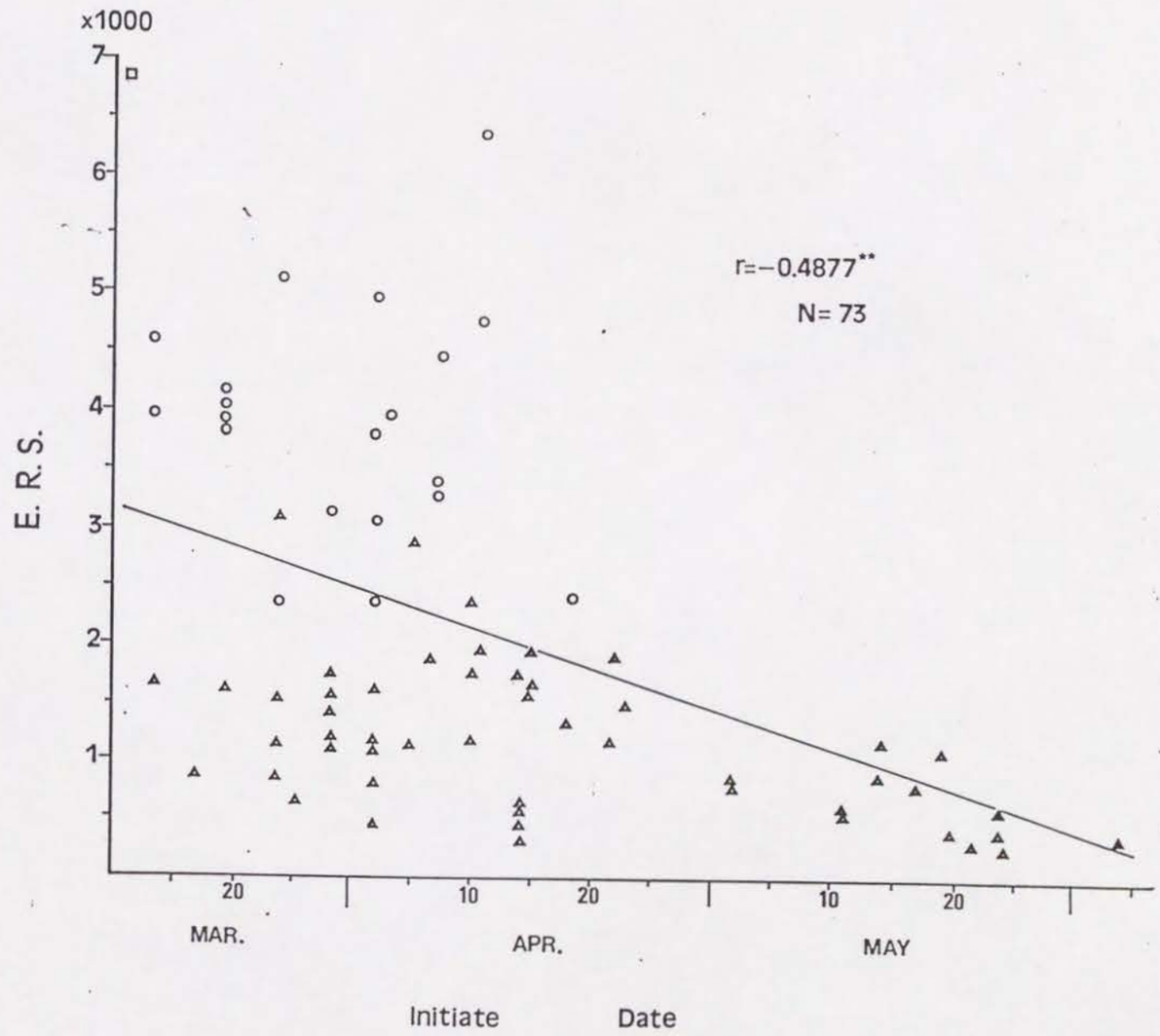


Fig. 33-a

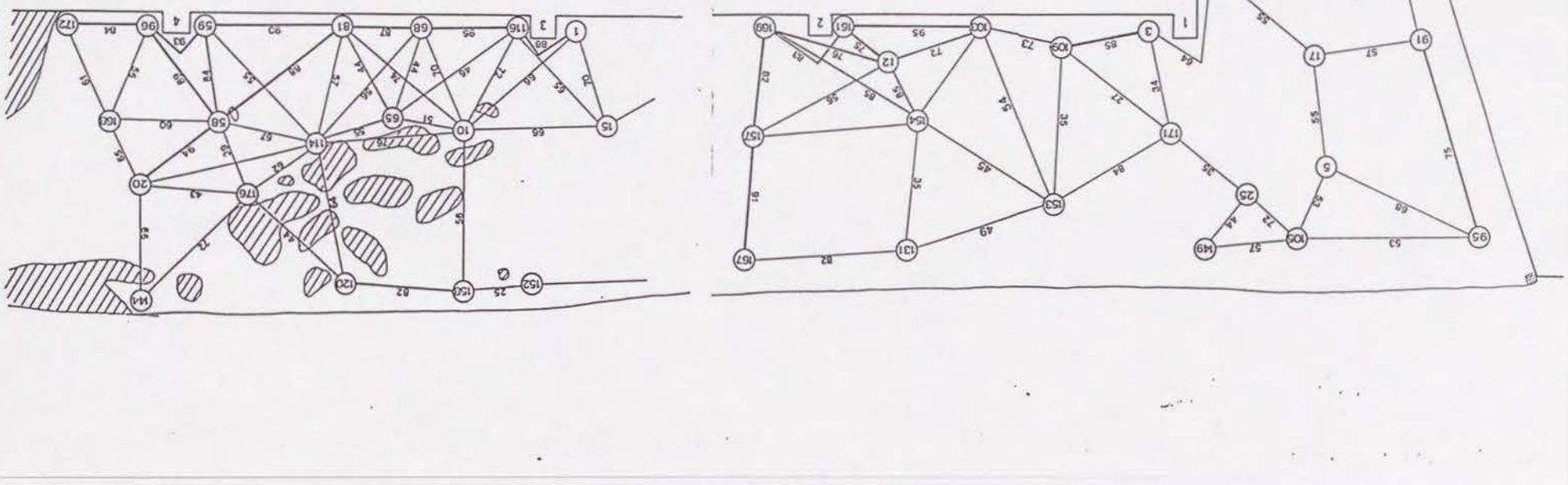


Fig. 33-b

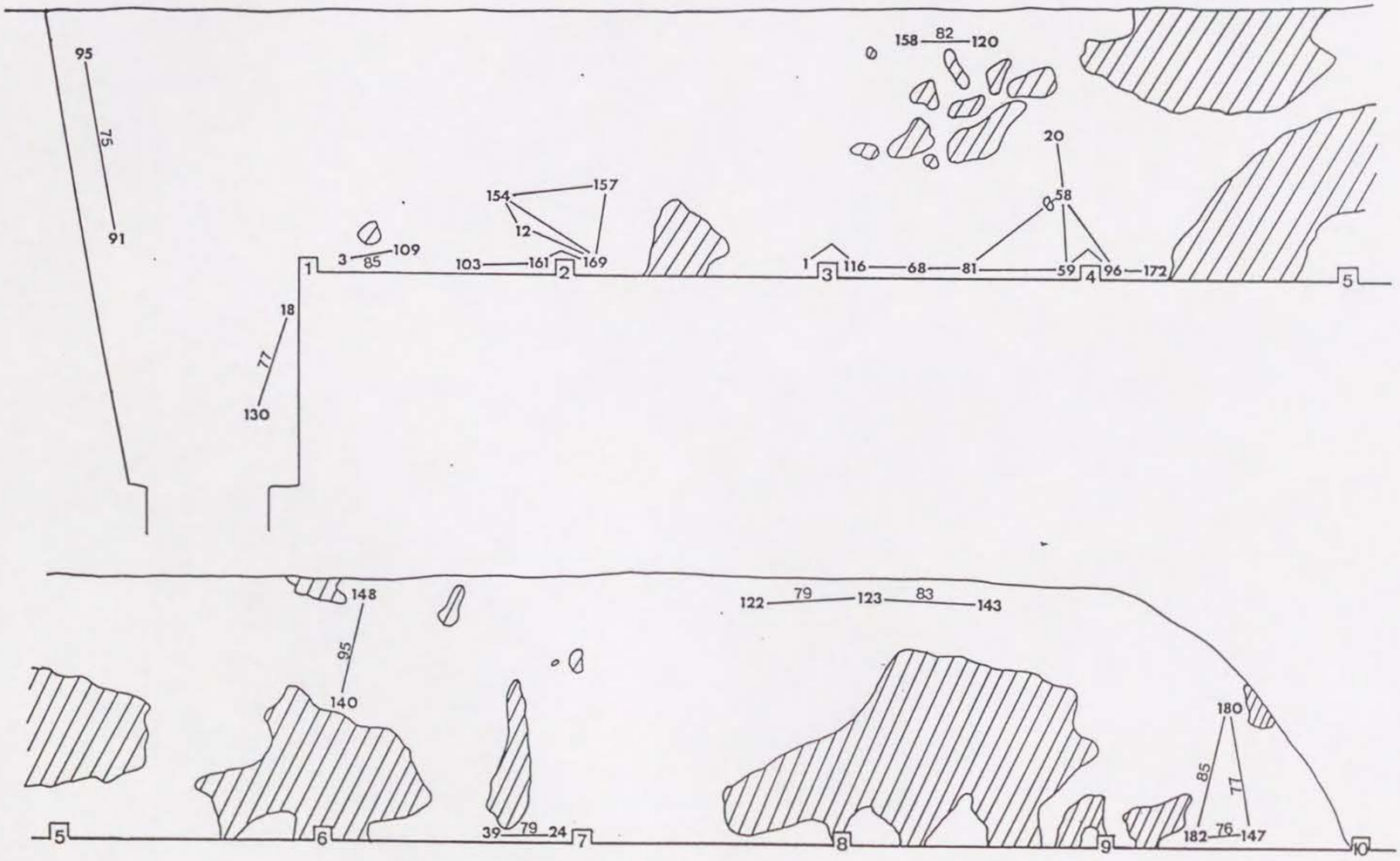
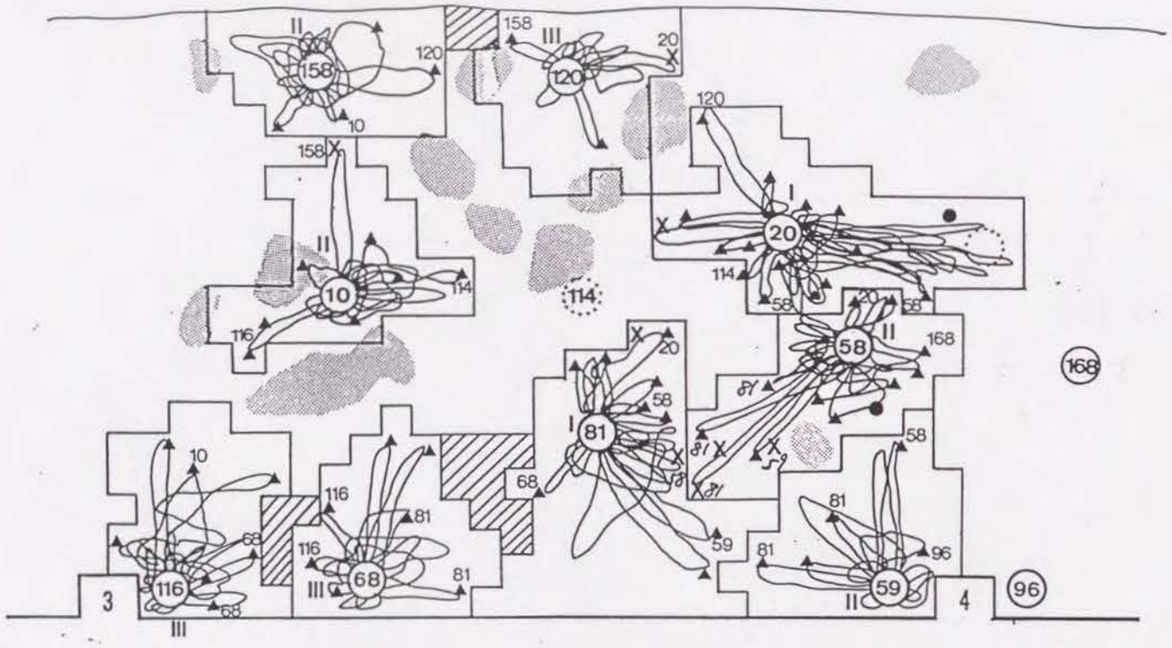


Fig. 34

May 3



May 5

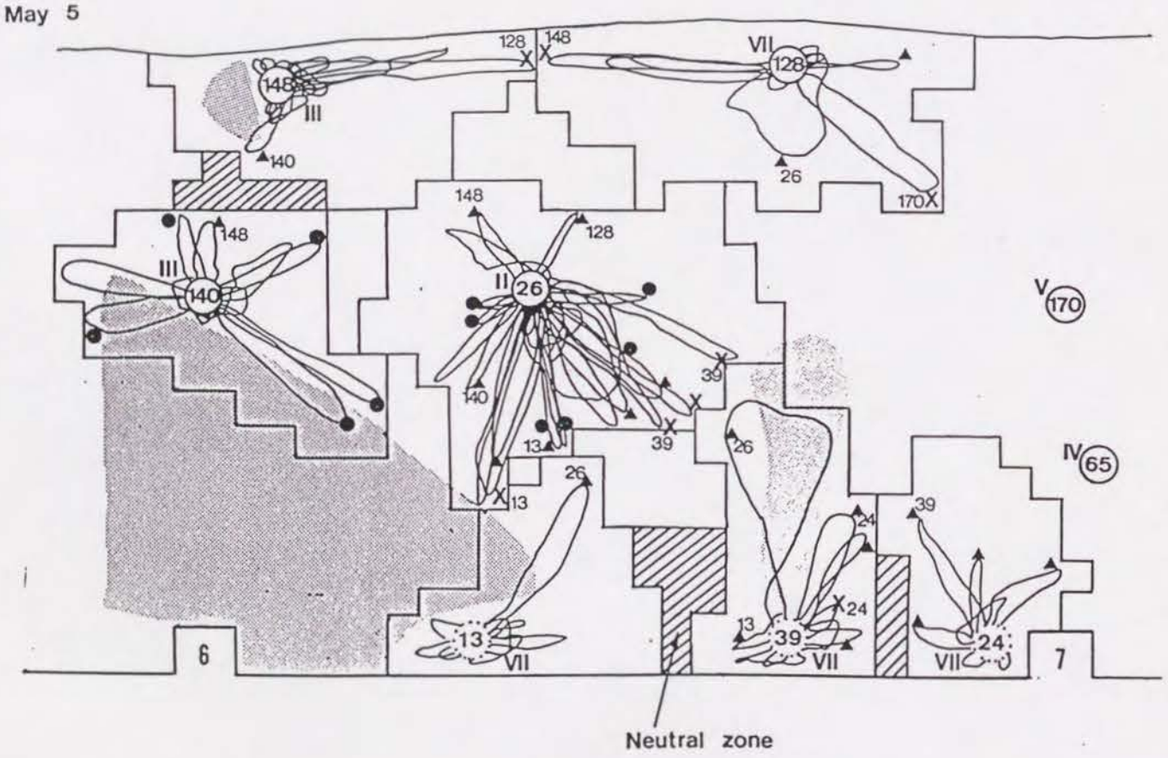


Fig. 35-(1)

Fig. 35-(1)

Nesting males in
Link J.

● : against link members

▲ : against non-link members

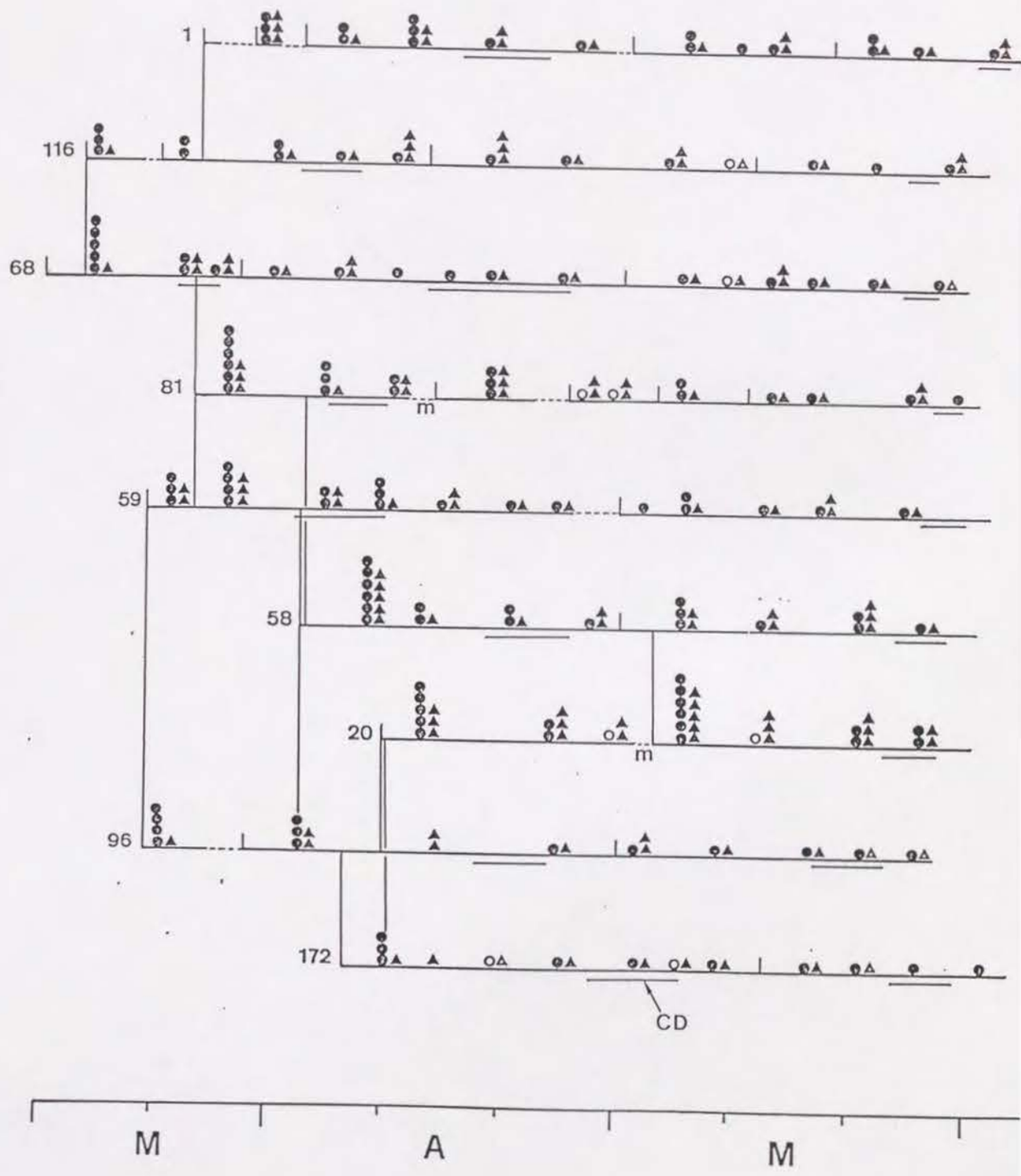


Fig-35-(2)

Fig-35-(2)

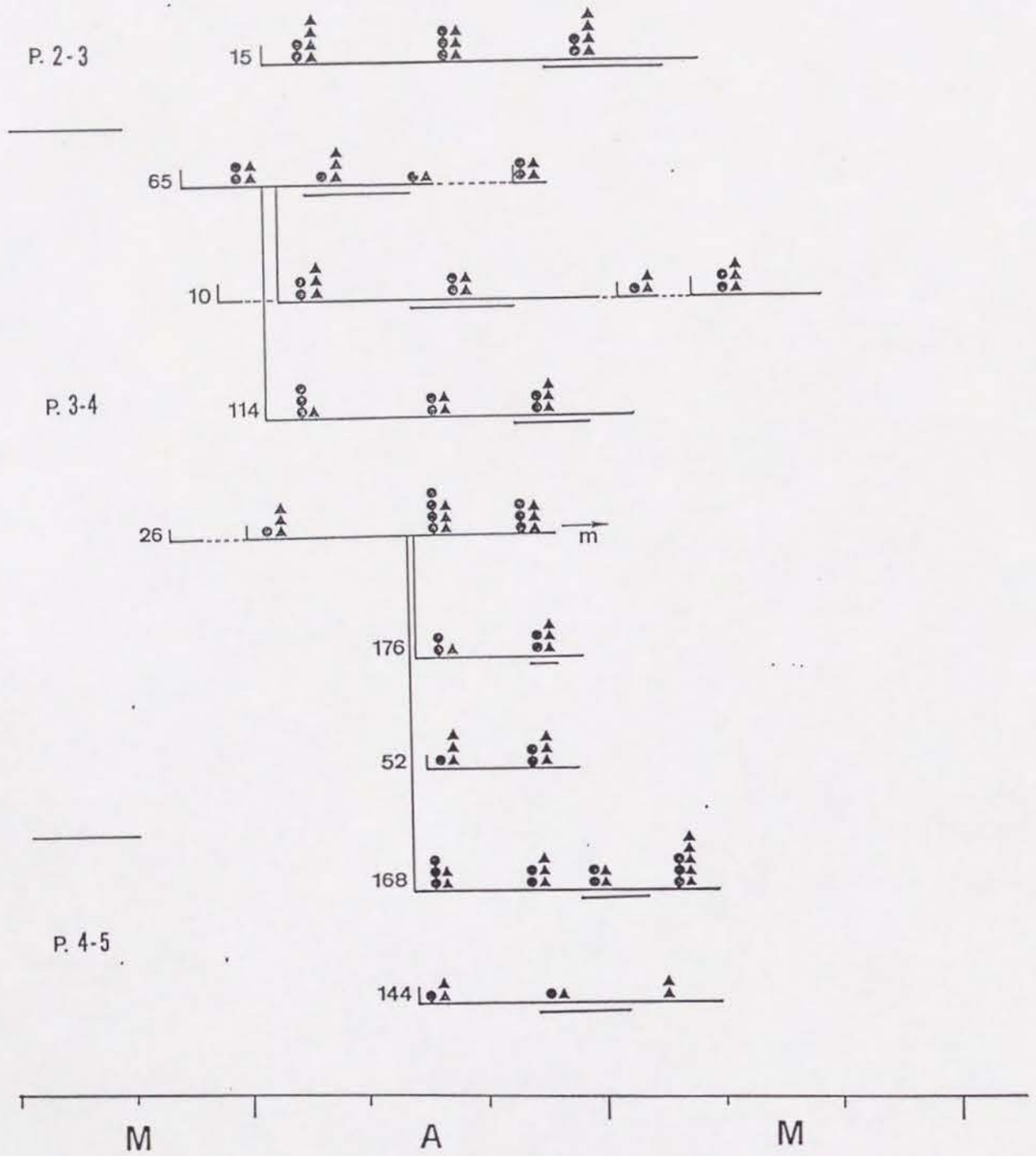


Fig-36

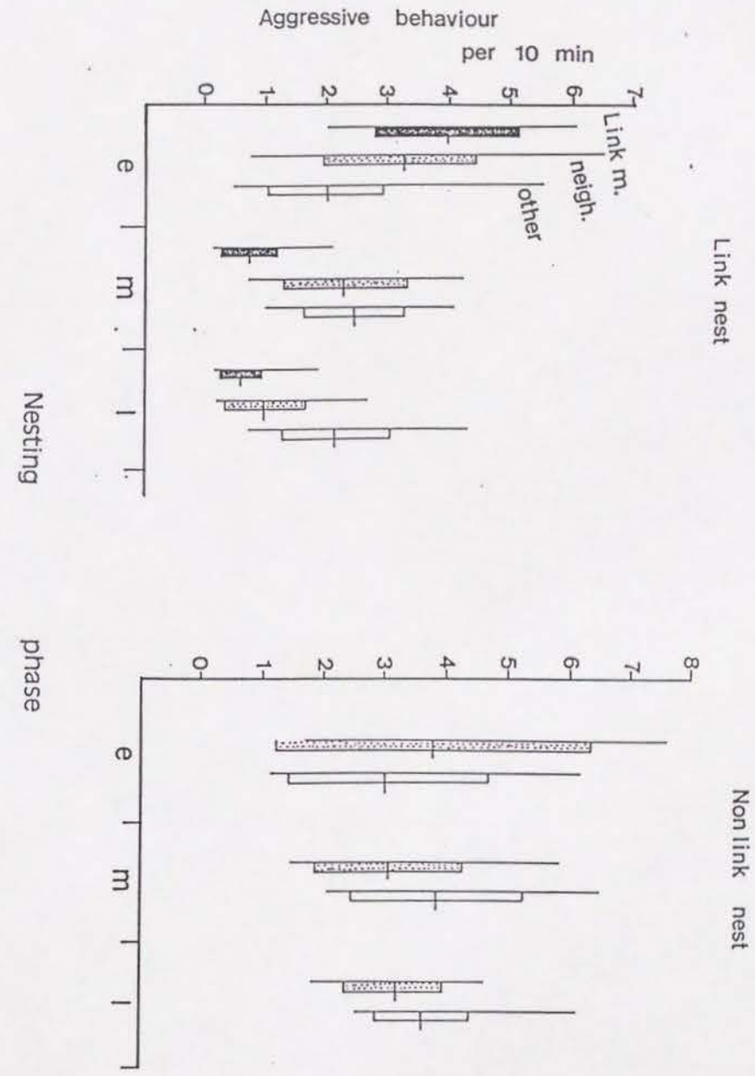


Fig-36

Fig.37(1)

●: Linkage members ▲: Non linkage members

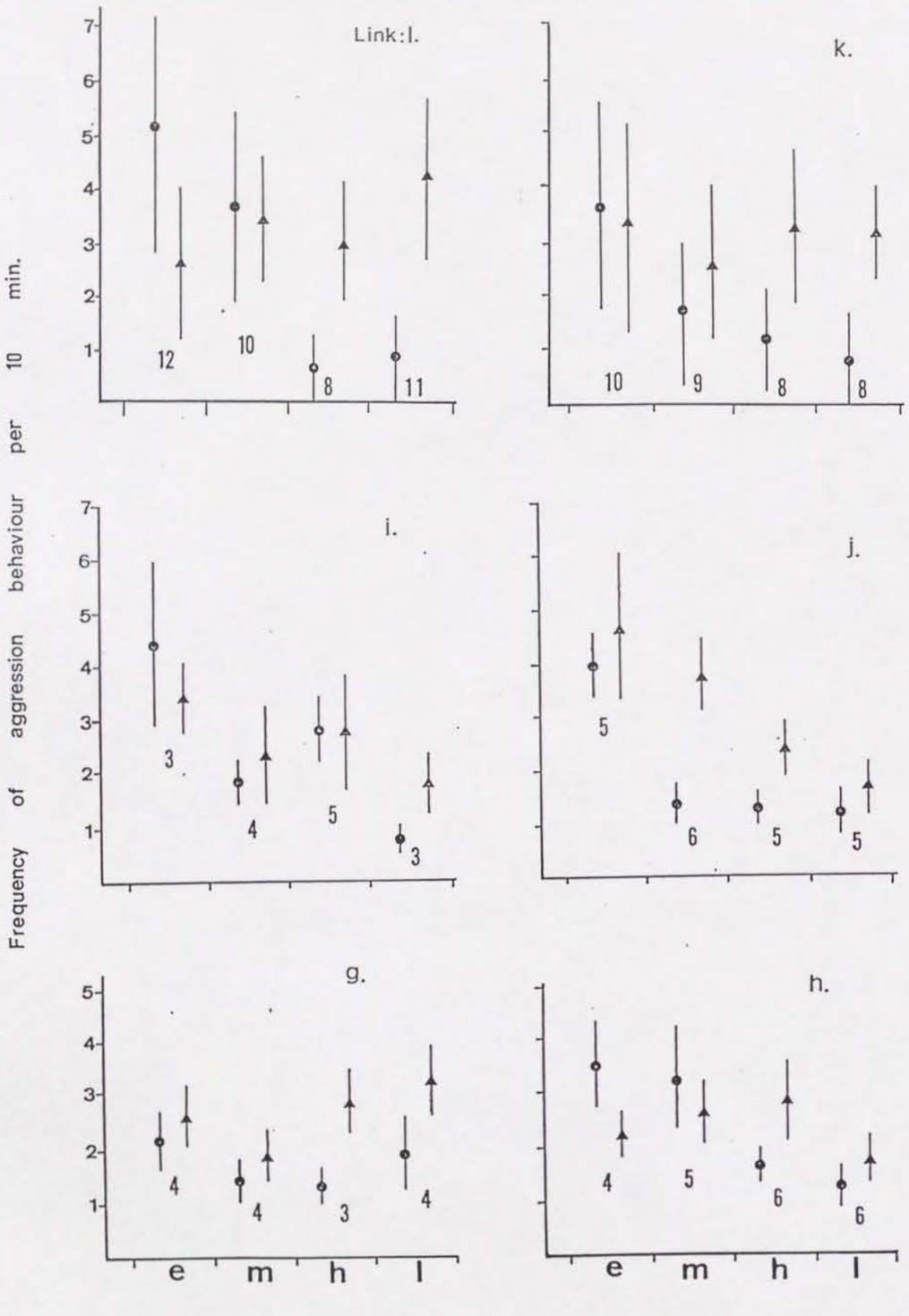


Fig.37(2)

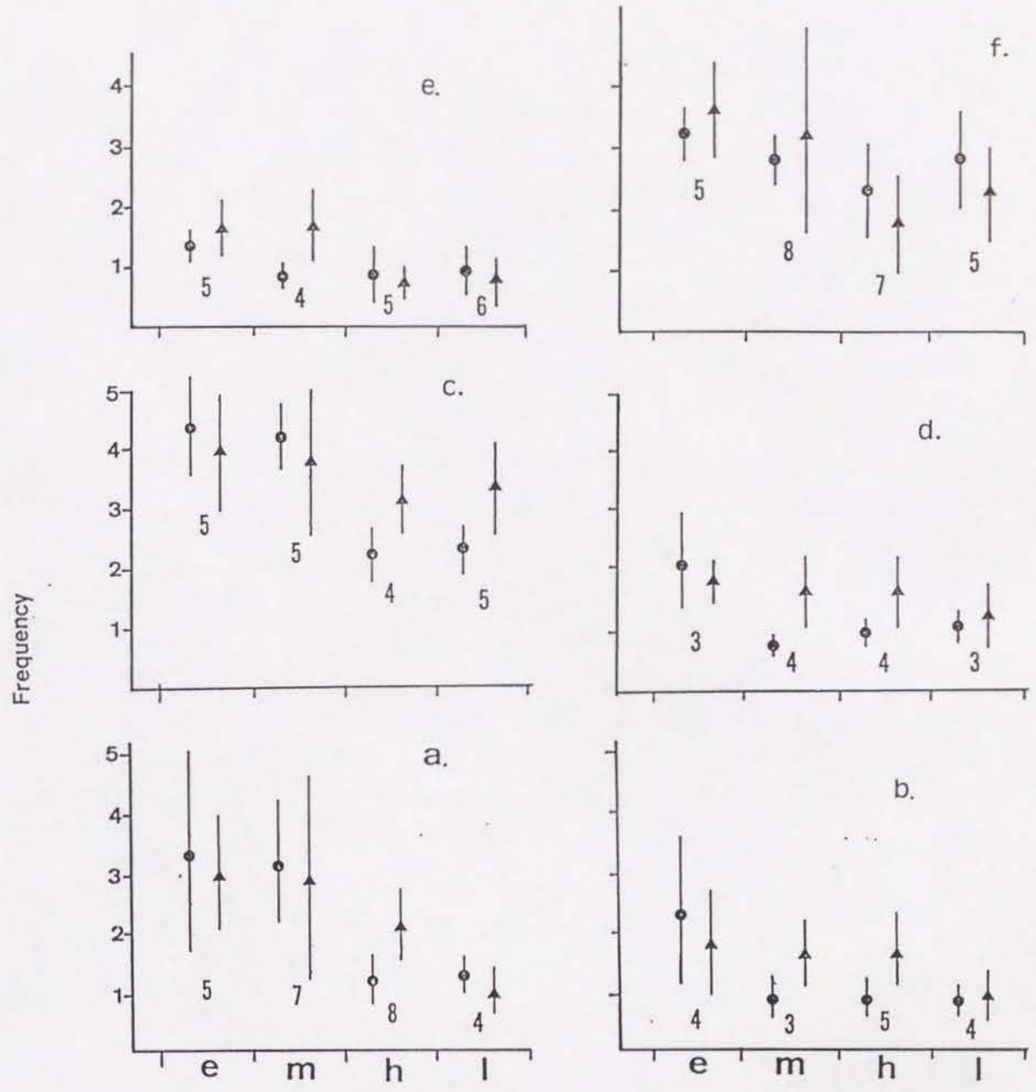
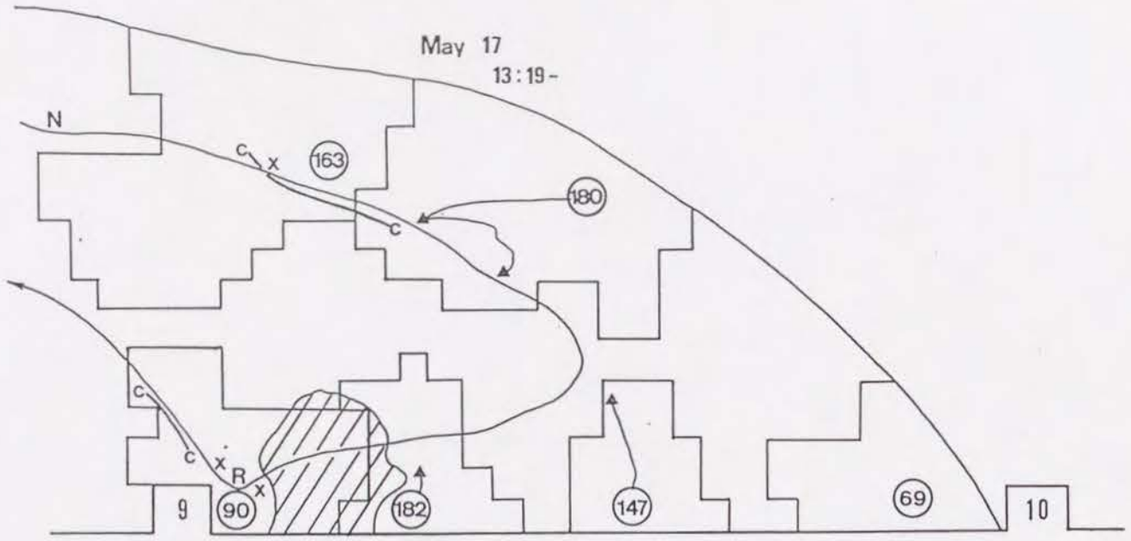


Fig. 38

A)



(B)

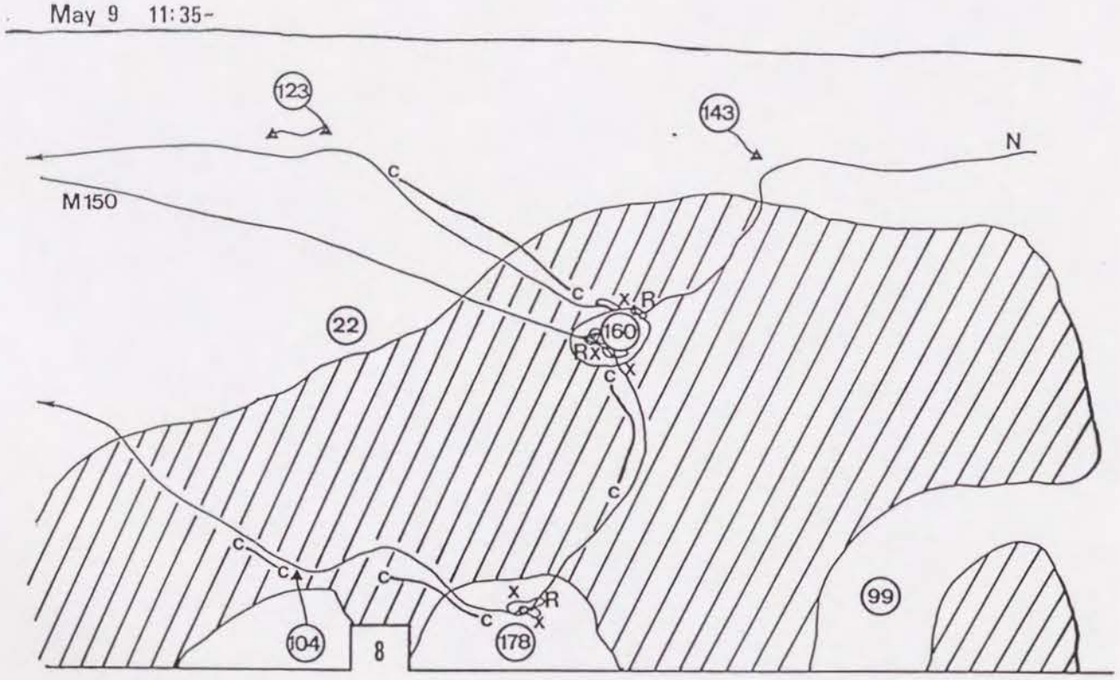


Fig. 39

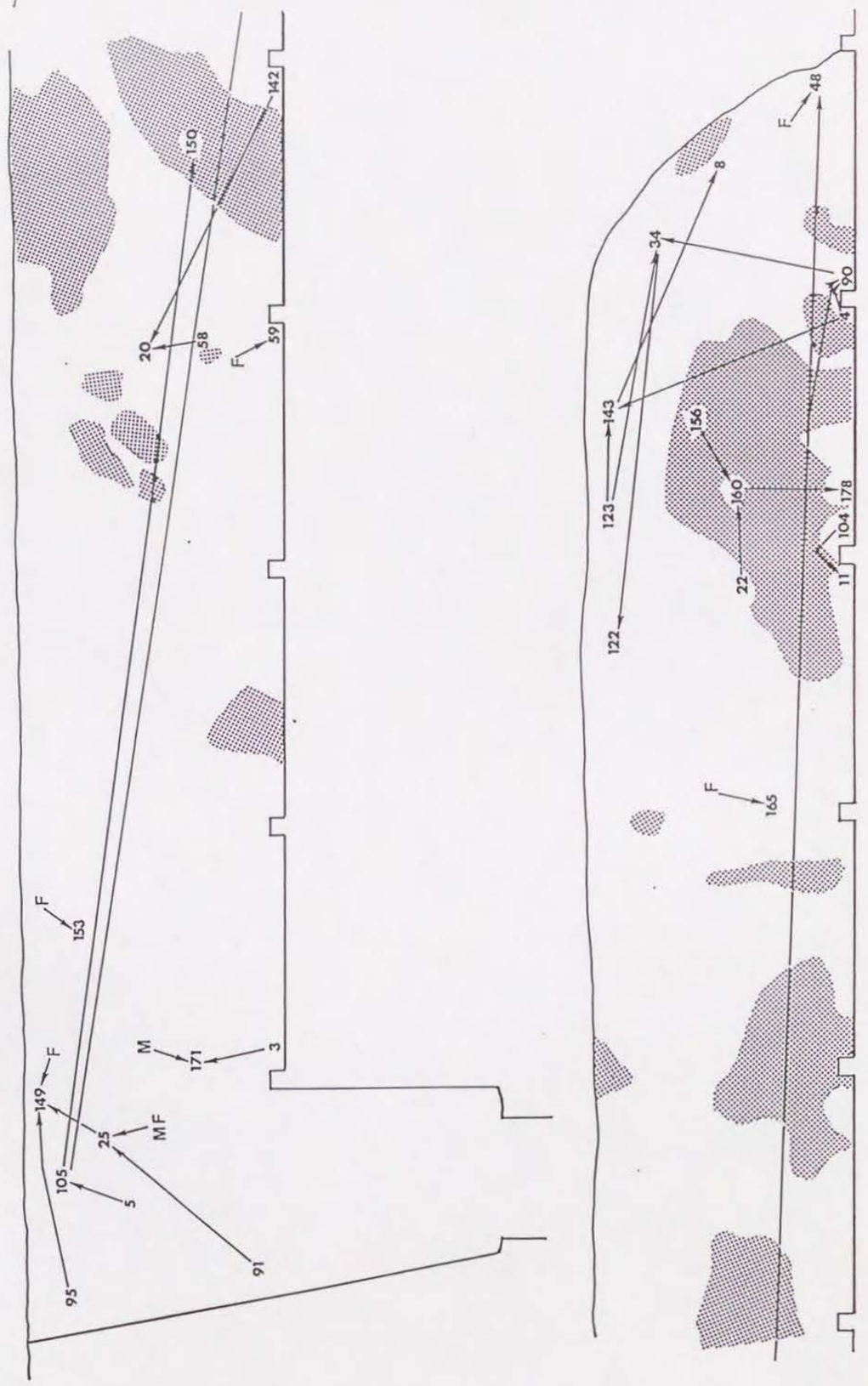
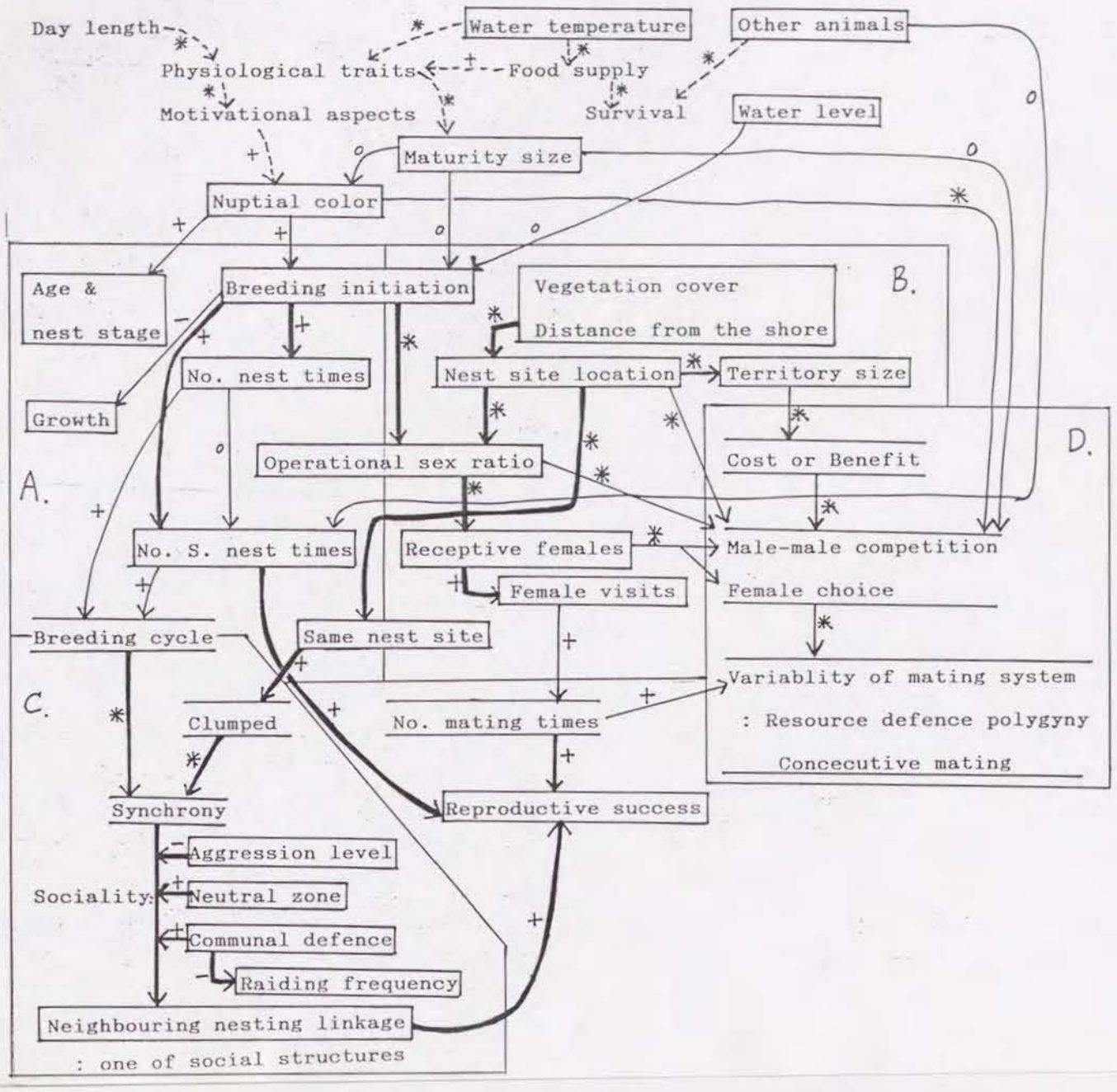
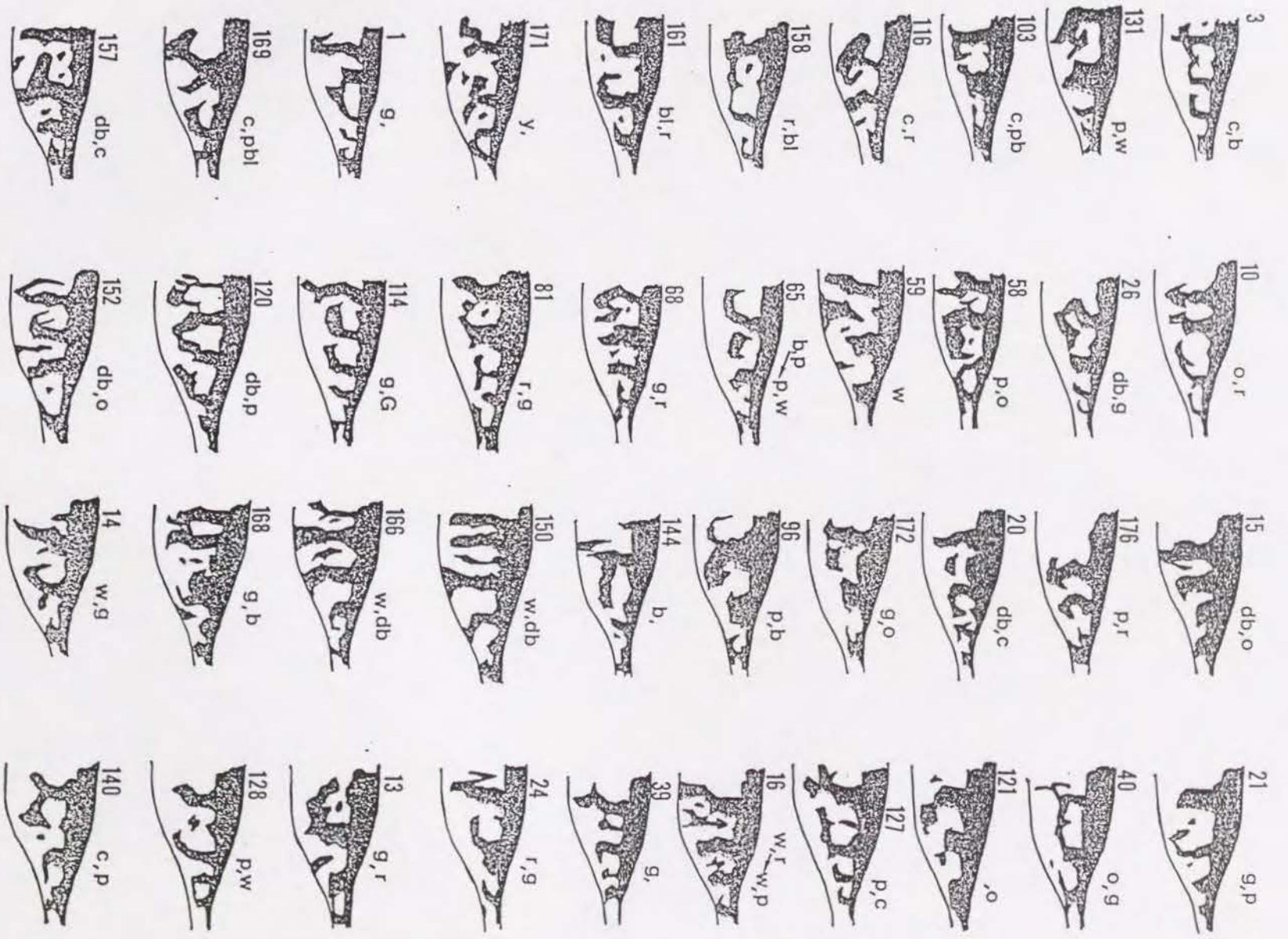


Fig. 40 Breeding structure of the three-spined stickleback.

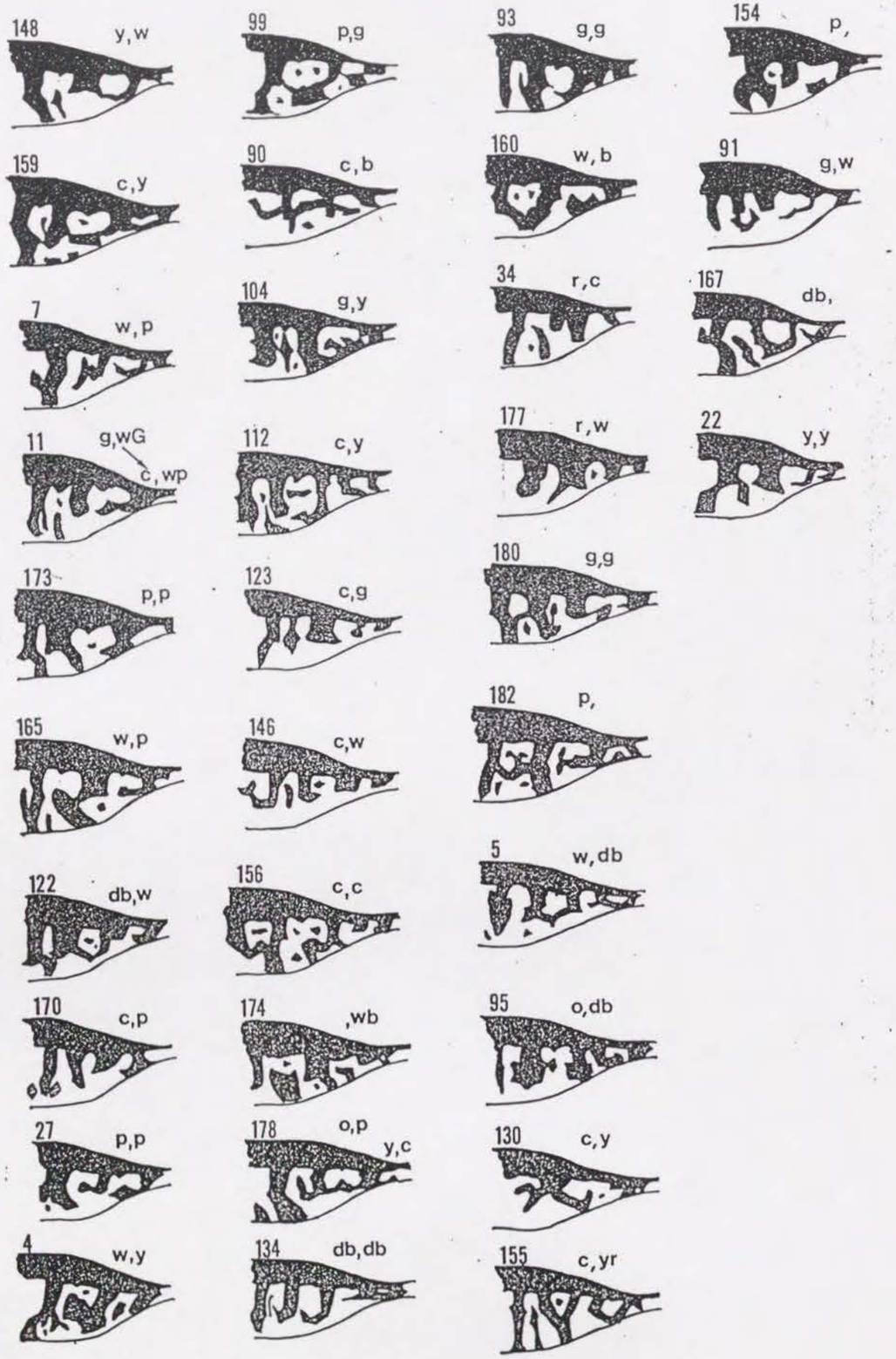


Appendix II.

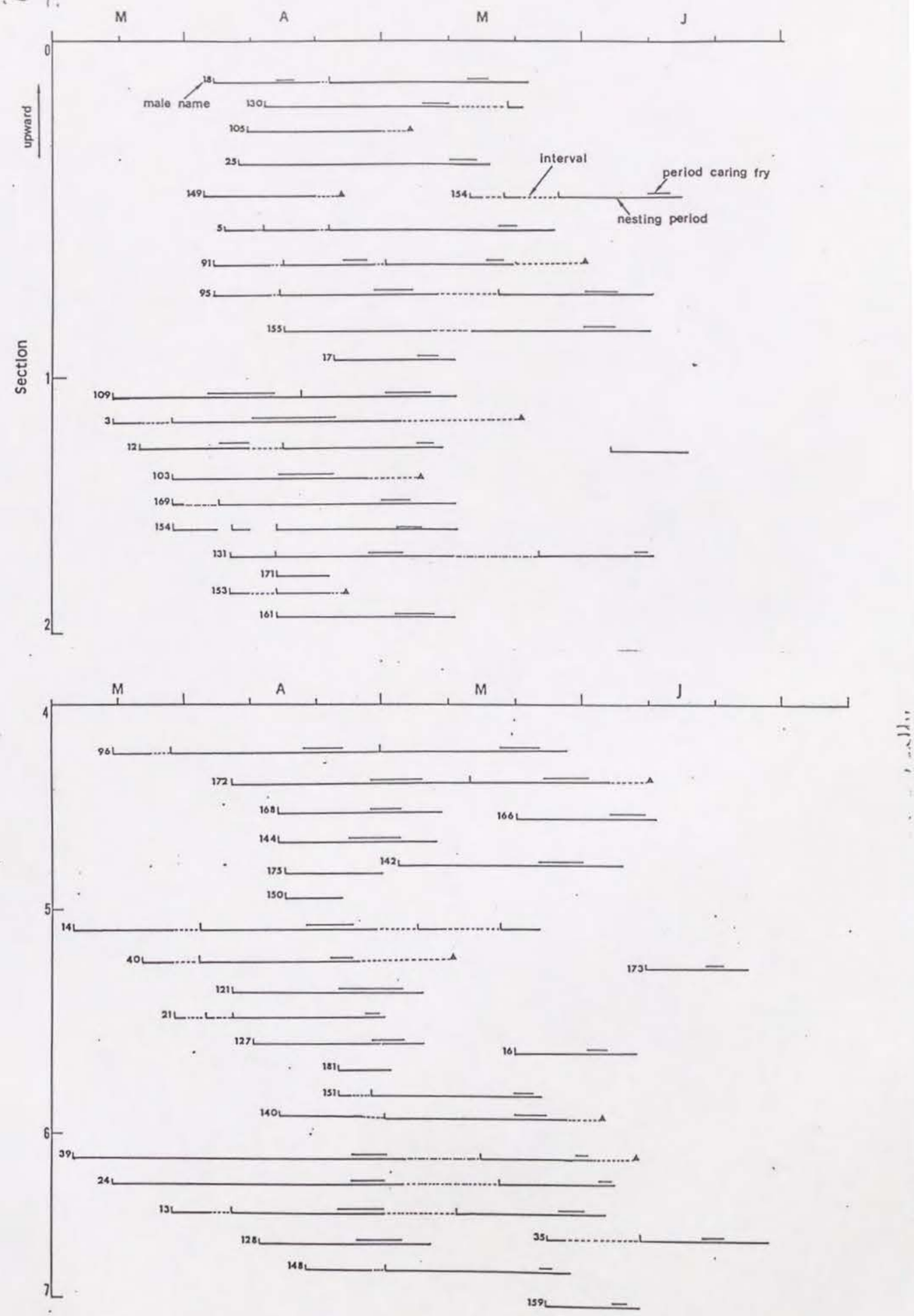


b:blue p:pink w:white db:deep blue c:spine cut
g:green r:red y:yellow G:gold
o:orange bl:black

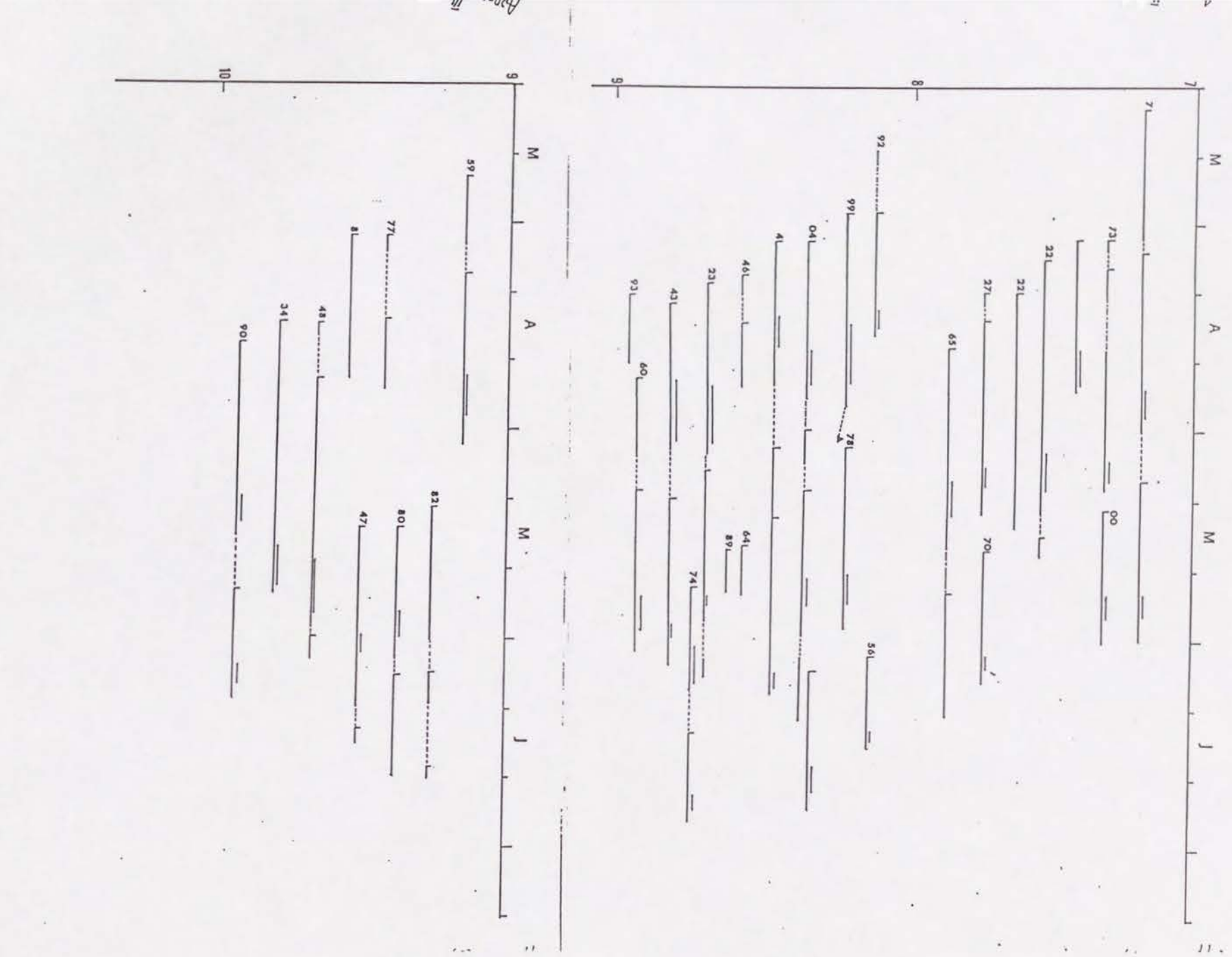
Appendix II continued



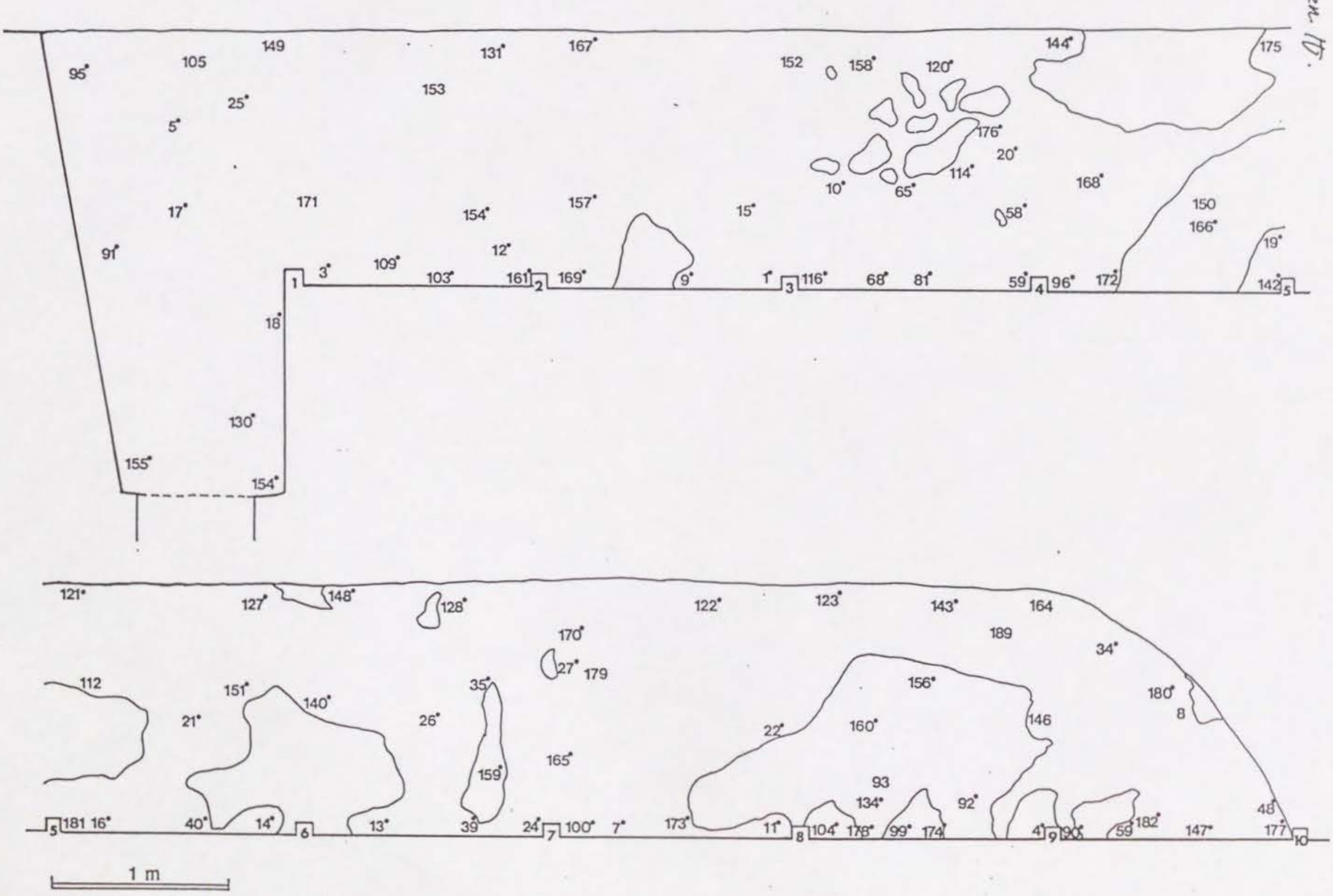
Appen. III-1.



Appen. III-2.



Appen. III.



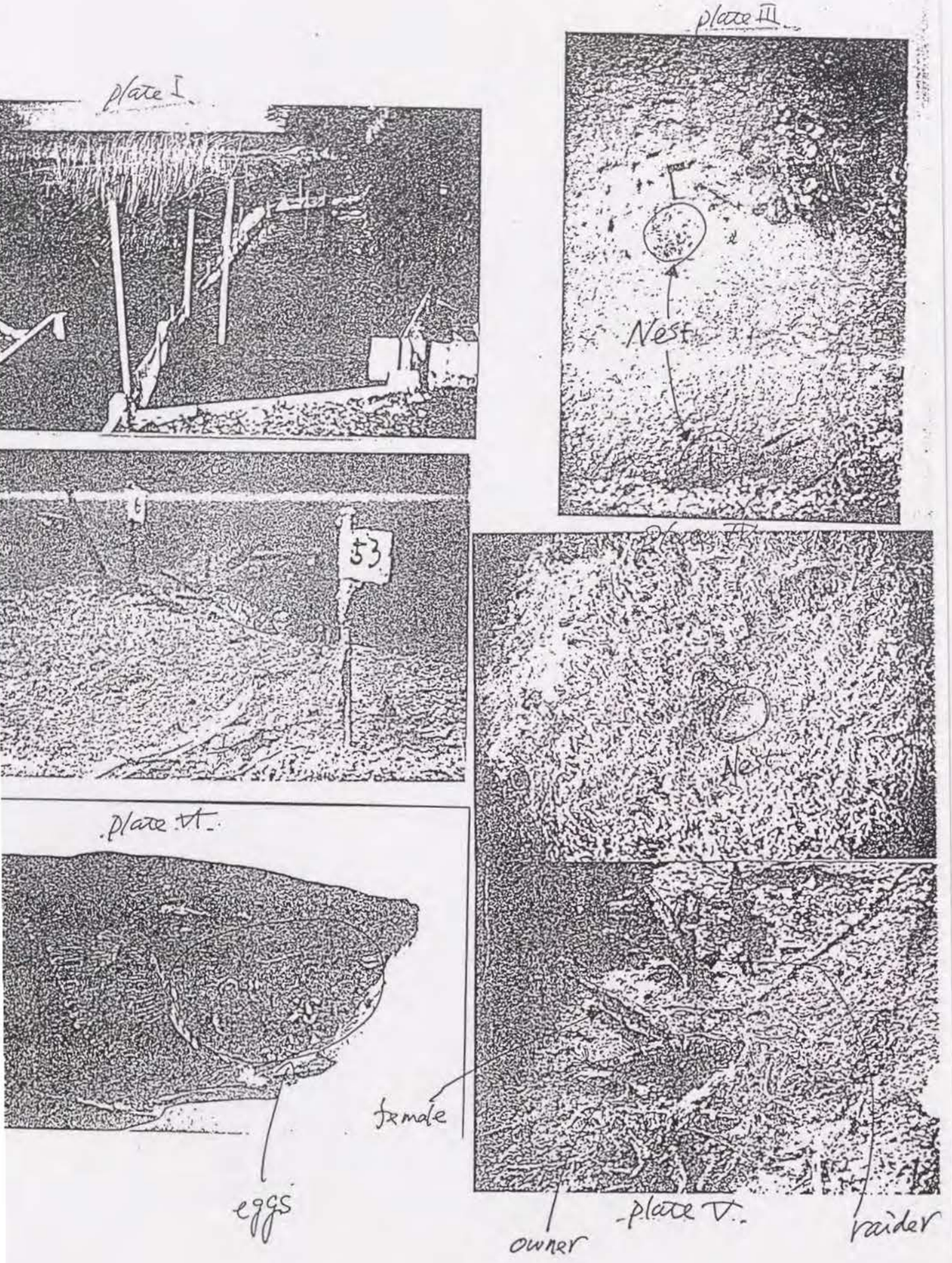


Figure description

Fig. 1 Maps showing locations of Central Japan, the Tsuya River and the study area. The Tsuya River flows from the Yohro Mountains (864 m at the highest peak) to Ise Bay. Broken lines in the middle map indicate gullies on alluvial fans.

Fig. 2 The study area which includes the study pool. The study pool which was enclosed by a net, is indicated by dots. Observations were usually made from the terrace.

Fig. 3 Water temperature in Tsuya River (black circles) and the inlet stream (straight line) during the study period in 1988.

Fig. 4 Fluctuation of water level during the study period. The sharp rise of the water level after late May was caused by heavy rainfall.

Fig. 5 Distribution of nests and vegetation growth during the study period.

Fig. 6 Measurements of nest site location. Black circles: active nests, DN: distance from the nearest nest, DS: distance from the shore. Aquatic vegetation, mostly *Elodea*, is distributed in dotted areas. The 50 cm radius of the nest was used when the proportion cover and the nest density were measured.

Fig. 12 Swimming routes of females during 10 minutes observation periods at each date. Asterisk indicate shoals containing gravid and spawned females and/or non-territorial males. Equal numbers on different dates represent different females. Letter S indicates the nest where the females spawned.

Fig. 13 Number of newly built nests during the study period split into first, second, third, fourth and fifth nests. Black squares show the males newly nested for the first time at a given date.

Fig. 14 The number of newly built nests (shaded bars) and the number of newly successful nests (black bars) per 5 days during the study period.

Fig. 15 Proportion of newly built nests per 10 days to the total number of nests during the study period (PN per 10 day/TNN). Proportion of successful nests to the number of newly built first nests per 10 day (PSN/NN per 10 day).

Fig. 16 Cumulative increasing curves of newly building (open circles) and first egg-laying nests (squares), and change of the number of nests per 5 day (solid line).

Fig. 17 Temporal change in the number of nesting attempts per male related to the date of first nest-building initiation (D. N. I.). The season is divided into ten periods of 10 days. Numbers indicate the sample size.

Fig. 18 Correlation between the duration of the nesting period and the date of nest-building initiation. Lines join breeding cycle of the same individual.

Fig. 19 Correlations between the duration of the nesting period, the duration of nest-building and unmated period (NUD), and the period that the male cares for the fry (CD).

Fig. 20 Temporal change between RS-index and date of nest-building initiation (D. N. I.). Black circles: group nesting males, open circles: non-group nesting males. Lines join RS-indices of the same individual.

Fig. 21 Temporal change in the frequency of nests divided into three categories according to the distance from the shore. Numbers above each bar indicate sample size.

Fig. 22 Discriminant analysis by Mahalanobis' generalized distance between successful nests and unsuccessful nests on the base of four nest site variables: distance from the shore, distance to the nearest nest, cover proportion and territory size. There was no significant difference between the two fates of nest.

Fig. 23 Seasonal change of $I_\delta(s)$ / (2s)-quadrat size relation (Morisita, 1959) of the nest distribution (s: square size per quadrat.

Fig. 24 Frequency distribution of territory size of successful nests (S) and unsuccessful nests (U).

Fig. 25 Correlation between the duration of the nesting period and territory size (mean size during nest phases II-IV). Territory size is measured by a square of 25 cm2 meshes. Open: 0 % cover; intermediate: 25, 50 or 75 % cover; close: 100 % cover.

Fig. 26 Correlation (regression analysis) between interval days of successive nests and the duration of the parental phase of previous successful nests (black circles) and previous unsuccessful nests (open circles). *: $p<0.05$.

Fig. 27 Spatial shifts of nest site location of successive nests of individual males between the pile section 3-4. Male 26 moved his 3rd nest to the pile section 6-7.

Fig. 28 Theoretical nesting patterns predicted on the basis of the means of nesting cycle and interval days between successive nest-building initiations (black squares) and the minimal duration of the nesting cycle (black circles). Four examples were shown, small black squares: successful nests; small open squares: unsuccessful nests. Numbers indicate the identification of nesting males. Mean and s.d. indicate those of date of nesting-building initiation for all males.

Fig. 29 Nest-site types (A-F) classified according to the distance from the shore and the proportion cover around the nest.

Fig. 30 Temporal change in the frequencies of nest-site types per 10 day period during the study period. Numbers on the upper line indicate the sample size.

Fig. 31 Fluctuation in territory size of males which nested more than twice in different nest-site types. Nesting success was indicated by an accent.

Fig. 32 Effort for reproductive success (E. R. S.) per individual male as a function of date of nest-building initiation (D. N. I.). Square: male with three successful nests; circles: males with two successful nests; triangles: males with one successful nest. Line is the least square regression, including reproductive effort of all males. **: $p<0.01$.

Fig. 33 Nest locations (circles in which number of the male are indicated) and degree of synchrony rate (SR, see text; shown by numerals on the lines) between pile section 1-2 and 3-4 (a). The value of SR rate was indicated on the line. Thick and thin lines connect nests of link nesting males and non-link nesting malers, respectively. (b): overview of the different linkages in the study area. Shaded areas indicate vegetation.

Fig. 34 Swimming routes showing the presence of a neutral zone (shaded areas) between neighbouring males within the pile section 3-4 and 6-7. Roman numerals, black circles, black triangles and X mark indicate the nesting phase, points where the nest owner courted the female, attacking points and threatening points by the owner, respectively. Numerals adjacent to black triangle and X mark mean the opponent males. Dotted circles and small dotted areas indicate vanishing nests and vegetation, respectively. Indicated is the time at which the swimming route is registered for a 5 minute period.

Fig. 35 Temporal changes in the frequency of aggression during 5 min. observation periods of link nesting males (linkage J) and non-link nesting males during the study period. Open circles and triangles indicate that aggression behaviours are not observed at a particular recording date. Letter m indicates the movement of a nest site outside the former territory. Dotted lines indicate the interval period between successive nests. Caring periods for fry are indicated by thin holizontal lines. Vertical lines show the connection of nesting linkage among males.

Fig. 36 Comparisons of temporal changes in the frequecny (mean+s.d.) of aggression against Link m. (link members), neigh. (neighbours of non-link) and other (male floaters, raiders and females) between link nesting males and non-link. e: early phase, all nests in linkage were situated in the unmated period. m: middle phase, situated in the incubation period. l: late phase, situated after fledging of fry. These abbreviations did not accord with the nesting stages that were indicated in Figure 7, because in the present figure the nesting phase was together dealt with more than two nests and then a time lag of stages occurred among the nests.

Fig. 37 Mean frequency (+s. d.) of aggression of link nesting males against same link members and non-link members during the nesting period for the different link nesting (linkage name a-l, see Table 31). h: hatching phase, all nests in linkage were situated just after hacthing. Numbers in the figure show the sample size.

Fig. 38 Comparison of territorial defense against raiding males between linkage (Male 180, 182 and 147; A) and non-linkage nesting (B). Capital letters M and N indicate raiding males with and without the nest, respectively. Letter x, c-c, R and black triangles indicate a direct attack, chasing route, raiding success and threating, respectively.

ig. 39. Swimming directions of successful nest raids.
M: by non-territorial males, F: by females. Males with a nest are indicated by numbers. Indicated are the pile numbers along the shore. Shaded areas represent vegetation.

ig. 40 Breeding structure of the three-spined stickleback.
The thickness of a line represents a degree of relationship between breeding factors. A broken line indicate a predicted relationships. * mark: meaningful correlation, + mark: positive correlation, - mark: negative correlation, 0 mark: no corelation. A area: a temporal dimension, B area: on spatial dimension, C area: a social dimension, D area: interpretations according to theories of mating system.

Appendix I The effects of marking with different colored-tubbed on the nesting success. The color pattern had no effect on the success and unsuccess of nests.

Appendix II Sketch of the pattern of the black pigmented areas on the left lateral side of each marked males. Indicated were the color codes of the colored tubes attached to the first and/or second dorsal spine(s), respectively.

Appendix III Temporal nesting pattern of individual males.

Appendix IV The first nest location of each male identified by numerals. Successful nests were indicated by a dot on the numerals.

Appendix V Dendrogram for the cluster analysis of nesting synchrony (SR as the similarity). The vertical dashed line represents the division line for linkage nesting.

Plate I Study area of the inlet which was seen from the upper part.

Plate II Two males (Male 53 and Male 6) with a marked nest. (phot. by K. Tokuda)

Plate III Males nesting along the shore (Male 9) and in the open middle area (Male 15). A distance between Male 1's nest and Male 15' s was 57 cm.

Plate IV Male (Male 165) nesting in an area covered with *Elodea*.

Plate V Above male is about to raid the other's nest. He attempted to steal a fertilization during the other male's mating. (phot. by K. Tokuda)

Plate VI Eggs (47 eggs) in the oral cavity of a raider from which the opercle region was removed. The male was sampled from the inlet outside the study pool. Eggs were also found in the stomach, but not own because of no nest.

Table 1. Scores (I-VII) based on the combination of nuptial coloration of head and lateral side of body.

	Scores of nuptial coloration						
	I	II	III	IV	V	VI	VII
Head	No red	Reddish-brown	Reddish-orange	Bright -red	Bright -red	Dark -red	Dark -red
Lateral body	No blue	No blue	No blue	Light blue	Bright blue	Deep blue	Drab blue

Table 2. Correlation of water level change and nesting success in the enclosure pool. The water level was measured at each nest site location at nest stage II and IV.
Kendall test = 0.1667, $p>0.1$.

	Change of water level (cm)								
	0	5	10	15	20	25	30	35	40
Nesting success (%)	40.3	71.1	70	82.4	75	75	100	50	66.7

Table 3. Comparison of body length between early nest-building males (before April 10) and late nest-building males (after May 10).

Period of first nest-building	No. of males	Body length (mm) April 10	May 10	Mann-Whitney's p value
Before April 10	23	52.3+1.7	53.0+1.6	>0.05
After May 10	14	52.8+0.8	55.0+0.9	<0.01

Table 4. Comparison in the frequency of successful nests and unsuccessful nests at each body size (in mm). S.: successful, US.: unsuccessful. Mann-Whitney's test: Ucal=54.5, p>0.05.

	Body length (mm)										
	46	49	50	51	52	53	54	55	56	57	58
Number of S. nests	0	3	15	8	19	18	27	10	7	5	3
Number of US. nests	1	3	11	7	12	13	14	4	5	2	1
S. Rate (%)	0	50	57.7	53.3	61.3	58.1	65.9	71.4	58.3	71.4	75

Table 5. Concerning males with more than two successful nests, correlation between body length and the rate of successful nests (number of successful nests/number of total nests) at each body length (Kendall test: =-0.786, p>0.05), and the number of males nesting successfully more than two times at each body size.

	Body length (mm)								
	50	51	52	53	54	55	56	57	58
S. rate (%)	56	40	45	36	26	11	0	25	50
No. of males	8	6	7	6	5	2	1	3	2

Table 6. Temporal change of receptive behaviours in females. Denominator numerals show the total number of observations. Early: before May 8, late: after May 9. *: significant.

	Enter but spawning*	Simultaneous following*	Following to non-nest male*
early	2/21	5/52	1/52
	9.5 %	9.6 %	1.9 %
late	5/11	11/28	4/28
	45.5 %	39.3 %	14.3 %

Table 7. Nuptial coloration scores and dates of first nest-building initiation (per 10-days period) for males in the enclosure pool.

Colour score	March		April			May			June	Total
III	2	2	2	5	1	0	2	4	0	18
IV	1	2	10	16	6	0	2	0	0	37
V	9	8	9	8	1	2	2	1	0	40
VI	0	0	0	0	0	0	2	1	1	4

ANOVA: Fcal=3.001, $0.01<p<0.05$

Table 8. Individual nesting times and successful nests.
*: Proportion of successful males (as least one successful nest) with a given number of nesting times.

		Successful nests				Number of males	Number of nests	Total number of successful nests	c/b (%)	Successfu males* (%
		0	1	2	3	(a)	(b)	(c)		
	1	14	25	-	-	39	39	25	64.1	64.1
Nesting	2	1	22	13	-	36	72	48	66.7	97.2
times	3	0	11	7	1	19	57	28	49.1	100
	4	0	2	2	0	4	16	6	37.5	100
	5	0	0	1	0	1	5	2	40	100
	Total					99	189	109	57.7	84.8

Table 9. Correlation coefficients of the number of nesting times per male (N. T.) and the number of successful nesting times per male (S. N. T.) and date of first nest-building initiation (D. N. I.). $p<0.01$.

	Nesting times	S. nesting times	Date of initiation
N. T.	1		
S. N. T.	-0.0967	1	
D. N. I.	-0.3266*	-0.4223*	1

Table 10. Correlation cofficients matrix of days from the date of nest-building initiation to the first egg-laying date (NB-Sp.), nesting days (N. D.), days caring for fry (C. D.) and the date of nest-building initiation (D. N. I.).

	NB-Sp.	Nesting days	Care days	D. N. I.
NB-Sp.	1			
N. D.	0.8269**	1		
C. D.	-0.0799	0.3865*	1	
D. N. I.	-0.2789	-0.5404**	-0.5531**	1

*: $p<0.01$, **: $p<0.001$

Table 11. Correlation cofficients matrix of nesting days (ND), actual nesting days (AND), proportion of AND to ND (ANP) and date of nest-building initiation for successful nests.

	Nest days (ND)	Actual nesting days (AND)	Actual nesting proportion (ANP= AND/ND)	Date of initiation
N.D.	1			
A.N.D.	0.4711*	1		
A.N.D.				
A.N.P.	-0.7158*	0.2087	1	
D.I.	-0.4590*	-0.5064*	0.1124	1
mean	26.3	15.0	59.3%	42.0
S.D.	6.7	2.6	12.3	20.5

*: $p<0.001$, N=96

Table 12. Success rate of nests per 10-days period during the study period. No correlation between time progressing and success rate ($p>0.2$).

	March			April			May			June	
Number of successful nests	6	8	22	15	7	11	17	8	8	2	0
Total number of nests	13	20	38	26	16	17	21	13	29	10	1
Success rate (%)	46.2	40	57.9	57.7	43.8	64.7	80.1	61.5	40	20	0

Table 13. Nesting success of nests with different degrees of cover within a 30 cm radius of the nest. Number in parenthesis represnts percentage.

Cover (%)	Successful nests	Unsuccessful nests	Total	
0	7(50)	7(50)	14	
25	24(61.5)	15(38.5)	39	
50	28(50)	28(50)	56	X^2_{cal} =8.081
75	28(66.7)	14(33.3)	42	p>0.1
100	8(80)	2(20)	10	

Table 14. Correlation cofficients matrix of variables associated to nest site location: nest density within a 50 cm radius of the nest, distance to the nearest nest, distance from the shore, cover and territory size.

	Nest density	Distance to the nearest nest	Distance from the shore	cover (%)	Territory size
N.Den.	1				
D.N.N.	-0.3762***	1			
D.S.	0.1063	-0.1495	1		
Cover	0.0459	0.2365*	-0.3668***	1	
Te. size	-0.2516**	0.0955	0.0933	-0.4883***	1

*: p<0.05, **: p<0.02, ***: p<0.001

Table 15. Correlation of nesting success and distance to the nearest nest. Mann-Whinety's U-test: P>0.2.

	Distance to the nearest nest (cm)									
	1 - 10	10 - 20	20 - 30	30 - 40	40 - 50	50 - 60	60 - 70	70 - 80	80 - 90	150-
Successful nests	1	3	24	27	27	9	0	0	0	0
Unsuccessful nests	0	2	17	14	15	11	1	1	1	1

Table 16. Correlation between nesting success and nest density within a 50 cm radius of the nest. Mann-Whinety's U-test: P>0.3.

	Nest density						
	1	2	3	4	5	6	7
Successful nests	20	32	23	12	5	2	1
Unsuccessful nests	17	19	14	12	3	1	0

Table 17. Correlation of the nesting success of successive nests.

		Latter nest: Successful	Latter nest: Unsuccessful	
Former nest	Successful	24	19	
	Unsuccessful	35	14	$X^2_{cal} = 3.002$
				$p>0.3$

Table 18. Correlation between nesting success and the number of previous nests.

	Number of previous nests: 0	1	2	3	4
Successful nests	59	42	15	1	1
Unsuccessful nests	46	21	7	4	0

Kolmogorov Smirnov Test:

Dr=0.0547 p>0.1

Table 19. Nesting days after the fry fledged, represented equal marks, per nesting pattern: once, twice or three nesting times. 1-2-3 shows the first, second third nesting. Letter S, U and N mean successful nest, unsuccessful nest and a case of non-nest building, repectively.

Nesting pattern 1-2-3	Nesting days after fledging 0	1	2	3	4	5	6	7	8	9	10	16	No. of males	Mean+s.d.
S-S=N	2	1	6	3	3	1	1						17	2.7+1.6
S-S=S					1								1	
S-S=U			1										1	
U-S=N	1	7	1	3	2	2	1				1		18	2.9+2.5
U-S=S		1		1									2	2+1.4
U-S=U			1	1	1	2		1					6	4.3+1.8
S=N		4	3	6	6	3	3	2	1				28	3.8+1.9
S=S		2	3	4	2	1	2		1			1	16	4.3+3.7
S=U		1	2	4	3	1	1						12	3.3+1.4

Table 20. Correlation between nesting success and the distance males move between successive nests. S: successful nest, U: unsuccessful nest, S-U: successful nest for the former and unsuccessful nest for the latter. X2cal=11.17, $p<0.05$.

Distance (cm)	Nesting success of the former and the latter nest S-S	S-U	U-S	U-U	No. of nests
0	13 (56.5%)	9 (45%)	8 (22.9%)	2 (14.3 %)	32
<30	7 (30.4%)	6 (30%)	17 (48.6%)	7 (50 %)	37
<100	1 (4%)	2 (10%)	6 (17.1%)	4 (28.6%)	13
>100	2 (9%)	3 (15%)	4 (11.4%)	1 (7.1%)	10
Average	31.7+59.7 (N=34)		53.5+98.2 (N=43)		

Table 21. Differentiation of nest-site types with respect to distance from the shore (+: along the shore, -: not) and degree of cover by algae (-: no, +: intermediate, ++: much).

Nest site type	Along the shore	Cover
A	-	-
B	+	-
C	-	+
D	+	+
E	-	++
F	+	++

Table 22. Correlation between coloration score and nest-site type of the first nest. ANOVA, $p<0.01$.

Nest-site type	Coloration score					
	III	IV	V	VI	Total	mean+s.d.
A	4	7	3	0	14	3.9+0.73
B	6	13	4	0	23	3.9+0.67
C	2	5	7	0	14	4.4+0.75
D	4	8	21	2	35	4.6+0.78
E	1	1	2	2	6	4.8+1.17
F	1	3	3	0	7	4.3+0.76

Table 23. Success rate (%) at each nest-site type related to the number of nests per male. In each all the proportion of successful nests to total number of nests is given. Number in parenthesis represents the sample size.

Nest-site Type	Number of Nesting times 1	2	3	4,5	Total success rate
A	50(10)	33.3(6)	42.9(7)	33.3(3)	42.3(26)
B	41.7(12)	42.9(14)	57.1(7)	-	45.5(33)
C	50(6)	60(15)	50(2)	20(5)	50(28)
D	70(10)	80(30)	56.3(32)	36.4(11)	63.9(83)
E	80(5)	75(4)	0(1)	-	70(10)
F	100(3)	100(4)	-	100(2)	100(9)

Total nest: $X^2_{cal} = 14.717$ $p<0.05$, Successful nest: $X^2_{cal} = 9.937$ $p<0.05$

Table 24. Correlation between caring for fry period (in day) and RS-index per nest for each nest-site type.
r = coefficient of rank correlation by Spearman,
* : group nesting at nest-site type D.

Nest-site type	Caring for fry period	RS-index	r
A	6.6±2.1	3±0.6	0.139
B	6.6±2.8	2.9±1.0	0.705
C	5.6±2.7	3.1±1.3	0.349
D	6.1±2.8	3.6±1.4	0.647
E	3.9±1.4	2.1±1.1	0.828
F	6.1±2.3	3.3±1.1	0.725
D*	6.4±3.0	3.9±1.6	0.704

Table 25. Nesting days, territory size and reproductive effort (see section 5-3 in the text) between successful (S) and unsuccessful (U) nests in each nest-site type. For each all the mean and s.d. are given.
*: $p<0.05$ (Mann-Whitney's U-test) for territory size.

	Sample size		Nest days		Territory size		Reproductive effort	
	S	U	S	U	S	U	S	U
A	11	16	28.3	9.6	45.7	44.5	1396.7	419.8
			4.8	6.7	12.0	10.5	375.1	300.9
B	15	18	26.6	9.8	54.5	42.9*	1466.2	483.8
			5.6	10.2	13.0	11.7	462.0	669.0
C	14	13	25.7	7.9	34.6	41.0*	907.2	306.8
			5.2	5.7	10.4	18.6	341.6	235.6
D	53	30	27.5	8.2	29.8	34.4*	832.0	274.3
			8.2	6.0	8.0	10.7	369.2	212.8
E	7	3	19.6	8.7	23.1	32.0*	458.7	308
			3.9	5.1	4.6	15.1	152.5	224.5
F	9	0	24.1	-	26	-	636.9	-
			3.9		6.6		231.8	

Table 26. Nest-site types of successive nests. Numerals represent percentage at each the nest-site type of the former nest.

		Later nest						
		A	B	C	D	E	F	Total
Former nest	A	77.8	0	0	22.2	0	0	9
	B	0	50	16.7	16.7	8.3	8.3	12
	C	0	0	72.7	27.3	0	0	11
	D	2.4	0	4.9	87.8	2.4	2.4	41
	E	0	50	0	0	50	0	2
	F	0	0	40	40	0	20	5

Table 27. Differences in nest-site type of later nest according to the fate (S or U) of former nest. Nest-site type A and B = open areas, C and D = areas with an intermediate degree of cover, E and F = areas with much cover. Here, this data was combined because of small sample sizes from nest-site types E and F.

Type of former nest		Later nest: same type	different type	Total
A & B	S	7	0	7
	U	13	9	22
		69%	31%	
C & D	S	28	0	28
	U	24	3	27
		94.5%	5.5%	
E & F	S	1	4	5
	U	1	0	1
		33.3%	66.7%	

Table 28. Distance between successive nests in the same territory of successful former nest. Numerals in parenthesis show the percentage at each nest-site type.

Distance moved (cm)	Nest-site type of the former nest: A	B	C	D	E	F
0	2	3	1	21	1	2
	(22.2)	(15.8)	(8.3)	(47.7)	(100)	(40)
≦30	1	12	9	13	0	1
	(11.1)	(63.2)	(75)	(29.5)		(20)
≦100	4	2	2	5	0	1
	(44.4)	(10.5)	(16.7)	(11.4)		(20)
100<	2	2	0	5	0	1
	(22.2)	(10.5)	(0)	(11.4)		(20)
Total	9	19	12	44	1	5

$\chi^2_{cal} = 5.72$, df = 4, $0.2 < p < 0.3$

Table 29. Frequency of social interactions (±S.D.) during 10 minutes observations and RS-index at nest stages III-V of each nest-site type during the peak period (10 April to 10 May). Stay at nest means the percentage of time the male was within an estimated 10 cm of his nest.

	Nest-site types:					
	A	B	C	D	E	F
Aggressive behaviour*	4.3±3.1	4.6±4.5	2.9±2.6	2.9±2.5	1.2±0.9	0.7±1.1
Intrusion*	6.1±3.0	7.1±4.9	4.7±2.4	4.8±3.9	3.2±2.8	1.8±1.2
Female visits	1.2±0.5	1.9±0.9	1.5±1.1	1.2±0.5	0.9±1.2	0.9±1.3
Stay at nest*	54.5 %	62.1 %	68.2 %	72.8 %	81.4 %	86.5 %
RS-index	3.0±0.6	2.9±1.0	3.1±1.3	3.6±1.4	2.1±1.1	3.3±1.1

*: ANOVA, difference between nest-site types at $p<0.05$

Table 30. Incidence of raided nest at each nest-site type (χ^2-test, $p<0.001$).

Nest-site type	Number of raided nests	Total number of nests	%	Times
A	3	26	11.5	5
B	5	33	15.2	8
C	2	18	11.1	2
D	4	83	4.8	6
E	3	10	30.0	4
F	2	9	22.2	2
Total	19	189	10.0	27

Table 31. Individual RE efficiency (RS-index / log RE) of successful males, separated according to nest-site types. Three categaries were divided by the number of successful nests times. Statistical test was used ANOVA for once and twice successful nests between nest-site types and then there were signifiant differences ($p<0.01$).

Nest-site type	Number of males	RE efficiency (mean+s.d.)
A	6	0.941±0.187
B	5	0.995±0.145
C	10	0.978±0.342
D	20	1.174±0.314
E	6	0.891±0.334
F	5	1.319±0.209
A-D	1	1.892
B-B	3	1.349±0.271
C-C	2	1.851±0.525
D-D	11	2.040±0.311
F-F	1	0.855
F-D	2	1.877±0.284
D-D-D	1	3.125

Table 32. Comparison of nesting activity and nesting success between link nesting males and non-link nesting males. Mean synchr.: Mean of synchronization rate at each link. S: successful.

Group name & males	No. Males	No. nests	No. S.nests	No. of nests per male	No. of S. nest per male	S. nests %	Mean synchr. %
a:91,95	2	6	4	3	2	66.7	76.7
b:18,130	2	4	3	2	1.5	75	75.3
c:158,120	2	3	3	1.5	1.5	100	82.4
d:140,148	2	4	2	2	1	50	94.9
e:39,24	2	3	3	1.5	1.5	100	79.3
f:122,123, 143	3	6	4	2	1.3	66.7	80.1
g:180,182, 147	3	8	3	2.7	1	37.5	79.2
h:3,109 103,161	4	6	5	1.5	1.3	83.3	89.6
i:154,157, 161,169,12	5	7	6	1.4	1.2	85.7	81.7
j:1,116,68, 81,59,58, 96,172,20	9	26	18	2.9	2.0	69.2	91.2
Total	35	76	52	2.2	1.5	68.4	84.1
Non-group nesting males	67	117	60	1.7	0.9	51.3	46.3

Table 33. The correlation between RS-index and group size for group nesting males. No significant ($r = 0.170$, $p>0.1$). Group name was shown in Table 31.

	Group name									
	a	b	c	d	e	f	g	h	i	j
RS-index	3.5	3.3	4.4	2	4	4	2.8	4	2.7	3.8
s. d.	0.6	1.5	1.8	1	1.4	1.7	1.5	0	1.0	1.3
Group size	2	2	2	2	2	3	3	4	5	9

Table 34. Difference of successful nesting times between group and non-group nests within nest-site type D. *: $p<0.01$.

	No. of males	No. of nests	No. of nests per male	No. of Successful nests	No. of Successful nests per male*	Success rate*
Group nesting males	22	51	2.3±0.9	39	1.8±0.5	76.5%
Non group nesting males	22	47	2.1±0.9	23	1.0±0.5	48.9%

Table 35. Reproductive success of group nesting males in the different nest-site types.
*: $p<0.01$

	Nest-site type					
	A	B	C	D	E	F
No. of nests of group nesting males	9	12	89	43	-	-
% of total nests	11.8	15.8	11.8	56.6	-	-
No. of Successful nests	4	7	7	32	0	2
% of total successful nests	7.7	13.5	13.5	61.5	0	3.8
Success rate (%)*	44.4	58.3	77.8	74.4	-	100
RS-index*	2.8	2.9	3	3.9	-	1.5
s.d.	0.5	1.1	1.6	1.4	-	0.7

Table 36. Descriptions of the individual nesting raidings.
*: Shoal A, **: Shoal B (see section 6-9 in the text).

Date & time	Male who was raided the nest (nest-site type)	Raider(s)	Results
Apr. 16 11:42	149 (B)	6 females & 25, 95	pushing of nest and eating of eggs
Apr. 18, 11:25	105 (A)	5	pushing of nest and eating of eggs
12:28	153 (A)	5-7 females	pushing of nest and eating of eggs
Apr. 20, 9:15	25 (A)	91	stealing of eggs
9:21	25 (A)	3 males & 12 females*	pushing of nest and eating of eggs
11:04	171 (A)	2-4 males & 5-9 females**	pushing of nest and eating of egg (?)
Apr. 23, 16:27	150 (F)	105	approach, pushing of nest and stealing of eggs
Apr. 24, 13:03	34 (B)	90	pushing of nest
Apr. 26, 11:59	48 (D)	105	pushing of nest
12:07	48 (D)	105	pushing of nest
12:10	8 (B)	143	pushing of nest
12:17	90 (D)	105	approach
12:18	90 (D)	4	approach

Table 36.

(continued)

Apr. 28,	15:27	11 (E)	104	seizure of nest
May 5,	13:50	20 (A)	142	pushing of nest
	14:15	20 (A)	58	pushing of nest
	14:27	20 (A)	58	sneaking of eggs
	16:30	20 (A)	58	sneaking of eggs
May 8,	12:47	178 (E)	160	disturbing courtship
	14:01	48 (D)	3 females	pushing of nest
	14:02	59 (D)	2 females	approach
May 16,	12:59	160 (F)	22	sneaking of eggs
	13:01	143 (B)	123	pushing of nest
	13:04	34 (B)	123	approach
	13:05	143 (B)	4	sneaking of eggs
	13:26	143 (B)	4	approach
May 26,	12:43	122 (B)	34	pushing of nest
May 29,	12:10	165 (C)	1 female	pushing of nest
May 31,	11:06	160 (F)	156	being attacked (to interrupt ?)
	12:25	173 (C)	7	sneaking of eggs
	14:10	122 (B)	7	approach
Jun. 6,	14:26	156 (F)	134	pushing of nest (to sneak eggs ?)
Jun. 11,	12:45	180 (D)	112	approach

Table 37. The nest stages of raider's nest and raided nest at the time of raiding. *: Interval period between successive nests.

Nest stage	Raider's nest (%)	Raided nest (%)
Parental phase with eggs	10.8	30.3
Parental phase with fry	10.8	6.1
Sexual phase without eggs	45.9	57.6
Without nest*	5.4	0
Nest-building phase (Pre-nest)	2.7	0
After caring for fry	2.7	9.1
Non breeder	5.4	-
Female(s)	16.2	-
Number of raids	37	33

Table 38. The relationships between lifetime reproductive success (represented as individual RS-index) and breeding factors by Kendall's rank correlation and ANOVA (*). FNA: first nesting activity. ++: Significant correlation at $p<0.01$ level. +: Significant correlation at $p<0.05$ level. NS: not significant.

Factors with RS-index	Correlation
Date of nesting initiation	++
Nesting days	+
Actual nesting days	++
No. nest times	NS
No. Successful nest times	++
Female visit	++
Nuptial color at FNA	+
Body length at FNA	NS
Territory size of nest stage III at FNA	NS
Water depth at FNA	NS
Change of water level	NS
Cover vegetation at FNA	NS
Mean distance from the bank	+
Nest site type*	++ (Significant difference)
Linkage or not*	++ (Significant difference)